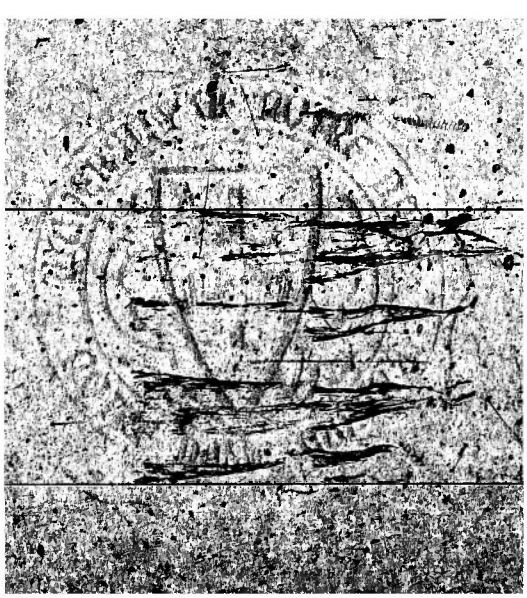
LOBUND - ONR
Semi-Annual Progress Report
Contract N6-ori-83, Task Order III

1 January 1953 - 30 June 1953



TO: Chief of Naval Research
Office of Naval Research
Department of the Navy
Washington 25, D. C.

ATTN: Microbiology Branch, Code 443

FROM: James A. Reyniers, Principal Investigator
N6-ori-83,Task Order III

SUBJECT: Enclosed Semi-Annual Progress Report (1 January 1953 to 30 June 1953). Contract N6-ori-83, Task Order III MR:131-067

DATE: 31 July 1953

Sir:

The LOBUND-ONR Semi-Annual Progress Report for the period 1 January 1953 to 30 June 1953 is herewith submitted.

In addition to maintaining schedules and programs during this period, we have been faced with reorganization of staff and routines in line with the NRC meeting on germfree life on 13 January 1953 in which Dr. Roger Reid and Captain C. W. Shilling participated. This effort has been aimed principally at production of germfree life. These changes are proceeding satisfactorily within the Institute, and we are ready for a limited expansion of equipment and personnel.

There is an immediate need to study the problems of shipping germfree animals and maintaining sterility of the environment. This is a major research project and must wait for cooperative action by the various agencies of the government acting through ONR to be activated.

The immediate future demands greater attention to technical problems and these will be emphasized. The work on large scale sterile chambers and other methods of entry than through a liquid trap are quite important to the military since, inaddition to the direct problem, the design and construction of protective garments and effective sterilization of these garments as well as environment seems to be much needed. There is a need for redesign of germfree equipment with the objective of making it more foolproof and automatic. Finally, there is a real need for a restudy of air supplies (in view of our losses due to water in the lines) and need for increasing the ventilation through the chambers.

During this period we successfully raised germfree turkeys (needed for antibiotic-growth studies) and resumed work on germfree dogs especially needed in shock studies.

The contribution to basic knowledge in nutrition, biochemistry, pathology, virology, immunology and bacteriology continues as seen in the various sections of this report.

Collaborative projects also continue with important new additions to the specific information sought. These projects unfortunately demand almost all our available facilities and the basic programs have been slowed down.

The problems encountered in rearing germfree animals are many and the work difficult at best but we feel that the technique is now of age. We have brought the work to function and it is recognized as basic to many problems in medicine and biology. The demands on these limited facilities continually increase, but this in itself is a healthy sign.

Respectfully yours,

James A. Reyniers

James A. Reyniers
Principal Investigator

University of Notre Dame

LOBUND Institute-ONR Semi-Annual Report

31 July 1953

For Period 1 January 1953 to 30 June 1953

CONTRACT: N6-ori-83, T. O. III NR:131-067

Prof. James A. Reyniers, Principal Investigator

Director, LOBUND Institute

Sections Reported

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I. ADMINISTRATIVE SECTION

FROM: R. F. Ervin, Assistant Director for Administration
(With the assistance of B. Terry and J. Mahon)

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report, 1 January 1953 - 30 June 1953

DATE: 31 July 1953

A. Task Personnel:

During the past six months the personnel situation continued as a problem in a small percentage of our total positions. The greatest difficulty has been stabilizing the staff which is responsible for feeding and caring for the germfree animals. Since we must maintain a 24 hour shift and a seven day week on this job, work schedules must include some evening time as well as Saturday and Sunday. By bringing one of our older employees, who has been on the night shift for three years, to the day shift and placing him in charge of feeding the germfree animal colony, it is hoped that we can straighten out this situation in a short time.

By the transfer of Mr. Pleasants from the biochemistry laboratory to chief of germfree mammal production, we have seriously reduced the manpower of our biochemistry section and to date have not been able to locate a replacement. The biochemistry staff has been further weakened by the resignation of one of our technicians who was performing assay work in this laboratory.

For the first time in many months, the diving personnel for the colony tank operation appears to be stabilized. We have been able to hire one of our former employees on a half-time basis while he attends Notre Dame during the next four years. He had previous diving experience with us before his induction into the Army.

Our bacteriology section has been strengthened by the hiring of Mr. Daniel Moran, a bacteriologist who has been placed directly in charge of the control work under the overall supervision of Mr. Wagner, our Chief Bacteriologist.

B. Physical Plant:

The new Roots-Connorsville Blower which will handle 200 c.f.m. at 7 pounds has now been installed and placed into operation. Steps are now being taken to connect this blower system to a dehumidification apparatus so that we can be assured of a constant dry air source. The constant increase in numbers of germ-free units in operation have taxed the original air supply and lead to one or two serious accidents wherein water got into the air lines.

Work is going ahead now toward the expansion of our

production section into an additional room wherein the standard "cage racks" are being installed to handle five or six additional germfree cages now being completed in our machine shop. This room will be used for hand feeding experiments and for experimental work on better interior cage equipment.

Facilities for handling dogs are being set up in our animal colony building. Our stockroom arrangements have also been improved so that glassware may be cleaned faster thus increasing the overall efficiency of our stockroom operations.

C. Major Collaborative Programs:

These are discussed in detail in the various research and collaborative sections of this report but are listed here as follows:

AEC (Advisory Committee) - Radiation injury
Army Med. Corps (Paul Gyorgy) - Liver necrosis
NIH (Floyd Daft) - Vitamin C.
NIH (W. H. Wright) - Amebiasis
Zoller (J. Roy Blayney) - Dental caries

D. Proposals and Contract Status:

Since our last report, Amendment Nos. 10 and 11 have been received, signed by the proper University authorities and returned to ONR Washington. These extend Phase III to 31 December 1953, Phase I to 30 June 1954 and Phase II to 31 December 1953. However, as a result of an agreement reached this past winter with ONR Chicago and ONR Washington, funds provided by any new amendment extends the termination of the entire Task Order a certain number of months based upon the percentage of the annual operating budget. Therefore, funds have now been appropriated for extension of the three phases of the Task Order to 30 June 1954. Since our proposals for each Phase are based upon a certain amount of work over a limited period of time (usually 12 months), it is understood that we are not obligated to work on the individual phases beyond the date provided by Phase money based upon the proposal. In the case of PhaseII, for example, wherein we requested support for work from 1 July 1953 to 30 June 1954 and only one-half that amount was transferred from AEC to ONR, we do not feel obligated to carry on work on this phase beyond 31 December 1953.

E. Government Furnished Equipment:

We wish to take this opportunity to express our appreciation to ONR Chicago for their excellent assistance during the past six months in obtaining some very badly needed equipment and material as GFE. Among the items already received or for which shipment orders have been issued are:

1 shipment Sheet Steel
1 shipment Bar Steel
1 shipment Glassware (miscellaneous)
1,105 c.f.m. air compressor

1 Hydraulic Shaper
1 Drilling Machine
1 Pyrometer
1 Ames Bench Lathe
1 Refrigerated Centrifuge
1 Small Carrier Air Conditioner

II. GERMFREE PRODUCTION, APPARATUS, AND TECHNIQUES

(Note: In March, 1953, a series of reorganizations began in the Germfree Animal Production Section of LOBUND Institute. Because of the increase in numbers of cages in use and numbers of animals raised along with the colony tank operations, growing complexity of experimental manipulations demanded, and the need for adding to the animal species produced, this reorganization was necessary. The first major step was a division of the Production section into "Operation" and "Mammalian Production". B. A. Teah remains in charge of Germfree Apparatus and Operations. He also retains supervision of germfree avian production. J. R. Pleasants was transferred from the Biochemistry Section to head the newly formed Germfree Mammalian Production Section. Reports from these two sections follow.)

A. Germfree Operations

FROM: B. A. Teah, Chief of Germfree Operations
(With the assistance of E. Zelmer, H. Thompson, B. Werner, J. Uselding, O. Youngquist, E. Snellgrove, H. Kennedy, P. Claxton, R. Terry, J. Timmons, and L. Terry).

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report, 1 January 1953 - 30 June 1953

DATE: 31 July 1953

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1. Apparatus:

The equipment used for this reported period was; 5 series 50 cages, 4 series 20 cages, 1 series C cage, 10 series 100 cages, 10 series 200 cages, 2 series Ex. cages, 1 X-ray cage, 1 diet cage, and 1 operating cage.

Since this equipment has not been added to since early in 1953, it might be well now to give the time they have been available for production and type of material from which they were fabricated.

4 series 20	Carbon Steel	1938
5 series 50	Carbon Steel	1946
10 series 100	Stainless Steel	1950-51
10 series 200	Carbon Steel	1950
1 series C	Carbon Steel	1940
1 Ex. cage	Stainless Steel	1947
1 X-ray cage	Carbon Steel	1951
1 Ex. cage	Carbon Steel	1951
1 Diet cage	Carbon Steel	1953
1 Operating cage	Carbon Steel	1950

The 4 series 20 cages will have to be replaced in the near future. The remainder of the equipment is in sound and usable condition.

We are now preparing a new cage room to house five new stainless steel cages. These will be similar to our 200 series cages with the exception that the glove rings will be increased to 8 inch diameter. These cages will be equipped and set up to handle only hand-feeding experiments. If the present progress continues at the same rate, these cages will probably be available for use in latter part of September.

In the past six months, we have encountered more glove trouble than we have at any other time, i.e., in the way of tears, punctures and weak spots. In the last progress report (1 June to 31 December 1952), it was reported that the all neoprene glove produced by Dewey & Almy seemed to be an improvement over American Anode gloves. But we encountered difficulty in getting enough gloves for our use. Recently, Dewey & Almy sold their glove equipment and formulae to another company which is not yet in production.

American Anode, supplier of our latex gloves, has been working on the production of all neoprene gloves. Up to now we have received only a few samples for trial. Preliminary tests indicate that this will be an improvement over the latex gloves.

2. Techniques:

(a) From the first of the year to the middle of April, a very large percentage of all chick experiments started were found to be contaminated from hatch (details will be found in Section IV). In most of these experiments, the contaminant was a pure culture of Pseudomonas. Change in source of eggs did not alter the results. A close inspection of the routine egg sterilization procedures used successfully formany years at LOBUND failed to account for this sudden increase in contaminations. It was then decided,to change the germicide from mercuric chloride to "Detergent Sanitizer No. 115", a compound produced by Rohm & Haas (Philadelphia) for the sterilization of egg shell surfaces. All evidence to date indicates that this change has eliminated contaminations from hatch.

(b) Culture tubes for testing sterility of cages were frequently becoming wet when passed through the sterile lock. To eliminate this, all cages were provided with small stainless steel containers, which will hold the test tubes and keep them dry. Also individually numbered containers for cultures transported to bacteriology was inaugurated, thus eliminating any chance of cage cultures being mixed.

(c) In the past six months we have attempted twice to rear germ-free dogs. The first was contaminated due to faulty operational techniques,-the second was germfree. For this type of experiment, a new interior cage was designed, basically one-half was allowed for run space and the other half both recessed and raised above the run area for storage of equipment.

(d) The eleven cages in the Biology Building have all been re-piped and new type filters added to all cages. Also as time permits we are re-piping all cages so that the air supply will come into the side, and not through the bottom of the cages. Tests show that this change will cause a lower humidity in the cages.

(e) An auxiliary compressor for operating the heating and air conditioning units has been installed. This is used entirely as a stand-by in case the older compressor should fail.

(f) The cooling ducts in the main rearing room have been relocated so that drafts directly over the germfree units are reduced.

(g) A new interior run cage, with a diet hopper to prevent wastage, mirrored stainless reflector to see all cage sections, and improved lids, is now being tested. Results will be given in a later report.

(h). A new Roots-Connorsville blower with a capacity of 200 c.f.m. was recently installed to replace the Nash-Hytor air supply. The Nash was water cooled, and several time flooded our air lines resulting in several contaminations to our rat cages. This new pump is air cooled and should give little trouble with water in the lines.

(i) Several Caesarian operations on guinea pigs were performed for the amebiasis project (reported in Section V).

B. Germfree Mammal Production

FROM: J. R. Pleasants, Chief of Germfree Mammal Production (with the assistance of W. MacAllister, L. Hary, M. Horton, L. Louis and M. Tarnow).

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report, 1 January 1953 to 30 June 1953.

DATE: July 31, 1953

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1. Germfree Rat Colony:

Germfree colony on Jan. 1, 1953 - 120 rats.
Germfree colony on June 30, 1953, 28 rats, 19♀, 9 ♂.

A total of 209 rats were weaning age or over during the report period, 101♀, 108♂.

Production:

46♀ bore 83 litters totaling 535 young, 6.4 per litter, 11.6 per ♀.
13♀ weaned 17 litters totaling 106 young, 6.2 per litter, 8.2 per ♀.

Uses:

41 germfree rats were assigned to experiments as follows:

No. Rats	Assigned To:	Use
1	LOBUND	Survey by Pathology
15	AEC	Radiation sickness study
6	U. of Pa.	Liver necrosis study
16	Zoller	Dental caries with controlled flora
3	N.Y.U.	Shock study by Zweifach

Losses:

A total of 141 rats were lost from the colony as follows:

35 died of two principal causes, lung affection due to overcrowded and moist living quarters, and volvulus of the intestine due to enlarged cecum.

3 presumed dead cannot be accounted for at the moment because of a change-over in the record system.

103 became contaminated. Approximately half the contaminations were due to mechanical failures, such as water in the air filters and clave door collapse. The other half were due to human failures, such as tears in the rubber gloves.

The contaminated rats were maintained in the apparatus for periods up to six weeks, and were disposed of for the following uses:

No. Rats	Assigned to:	Use
3	LOBUND	Evaluating effect of contamination on organs
13	AEC	Effect of monocontamination on radiation sickness
45	LOBUND	Serology study of increasing antibody production
10	LOBUND	Stock colony of close relatives of germfree rats.
12	Holtzmann	Parasite-free stock colony
20	LOBUND	Biochemical evaluation

A total of 429 pre-weaned young were lost by unexplained lactation failure, by failure to segregate the mother before parturition, andby generally overcrowded conditions.

The germfree rat colony showed a net loss of 92 animals during the period reported.

2. Hand-feeding Experiments with Rats:

Experiment 184 - Five rats started. Last two dead on 8th day.
Experiment 185 - Nine rats started. Last three dead on 12th day.

3. Hand-feeding Experiments with Dogs:

Preliminary experiments with conventional dogs indicated that puppies could be easily reared from birth, feeding sterilized milk formula (evaporated milk) in small nursing bottles fitted with premature baby nipples.

Two germfree experiments were started:

61C2-1 Five puppies were obtained by Caesarian section of a mongrel bitch. They appeared full-term, ate readily, and started to gain weight by the third day. They proved to have a single bacterial contaminant, and were removed for worm analysis. They contained the Ascaris, Toxocara Canis, in larval form.

61C2-2 Five puppies were obtained, one of which was immediately used to determine if worms were present. None were found. The four other puppies appeared premature, had difficulty in breathing and in eating, and all died in less than 24 hours. Their lungs and kidneys showed functional immaturity.

The two difficulties encountered above are being overcome as follows:

1. By timed breeding of the bitches used.
2. By maintenance of the pregnant bitch on a regimen designed to eliminate or greatly reduce her infestation by worms.

4. Changes Effected and Planned:

Two major changes have been made in the organization of Germfree Mammal Production:

(a) Animal care has been separated from equipment maintenance and operations.
(b) Colony care has been separated from hand-feeding.

(a) The first change is intended to permit a greater investment of time in the improvement both of animal care techniques and of equipment techniques. It has so far permitted a re-planning of the organization, a reorganization of the system for keeping records, and some time for redesign of interior cage equipment and diet experimentation.

(1) Records have been reorganized so that each rat's individual life history is kept up-to-date, both on the cage and in the office file. The cage copy is immediately available to any investigator who intends to use the rat.

(2) The present interior cage equipment is a compromise imposed by the large number of rats that had to be housed. Interior equipment is being designed for cages that will be devoted to reproduction. This equipment will:

i. Protect gloves by making it unnecessary to open the interior rat cage in order to feed, water, and clean the animals.

ii. Protect the health of the rats by providing more ventilation, and by preventing the spilling of water and feed.

(3) The present diet permits only a slow increase in the colony, because of the small percentage of rats weaned on this diet, both inside and outside the germfree equipment. Experimentation with autoclaved diets on conventional rat breeding, and evaluation of experimental diets occasionally used in the germfree colony, are in progress to find a diet which could be tried out on a part of the present germfree colony.

(b) The separation of colony care from hand-feeding is intended to improve both colony care and hand-feeding.

(1) The rapid turnover in feeder personnel, due to low wages and irregular hours, had put the care of the colony periodically in the hands of inexperienced and physically awkward personnel. Five or six persons had handled the colony feeding in any one week period, and no one had come to know the animals thoroughly. One fulltime man, with three years of experience in GF work, has now taken over colony care, relieved once a week by the head of animal production.

(2) Hand-feeding had to be sandwiched in between periods of colony care, and had been hurried in consequence. The present organization of the feeding staff permits the devotion of fulltime effort to hand-feeding.

C. Plastic Garment Sterilization

FROM: P. C. Trexler, Assistant Director for Research (with the assistance of Louise Reynolds)

TO: J. A. Reyniers, Director

SUBJECT: ONR Report 1 January 1953 - 30 June 1953

DATE: 31 July 1953

For sometime we have been trying to improve the dip tank entry to the germfree colony tank. Passage through the dip tank is tedious and requires considerable training. The hydrostatic pressure and buoyancy imposes limitations on suit construction. Since the germicide in the dip tank acts as a seal, it must be relatively stable. This imposes limitations since many potent germicides are unstable. At the present time the diver brings into the germfree tank a considerable amount of germicide which is undesirable as far as the health of the animals is concerned.

Most of the above difficulties can be eliminated if the suit can be completely sterilized by means of a germicidal spray or gas. There are several ways to make a gas tight lock for entry which will replace the sealing function of the dip tank.

In order to determine the practicality of gas or spray sterilization of a plastic garment, a plastic dry box was constructed of the same material, inoculated with spores, and then sterilization attempted by various means. Of the gases tried, formaldehyde seemed to be the best. However, it was difficult to remove from the plastic and required a prolonged contact in order to kill some spores. For these reasons it was not considered practical. On the other hand, of the liquid germicides tried, a mixture of 1% peracetic acid and Roccal seems entirely satisfactory. The most resistant spore found, Clostridium stereothermophilus, withstands a formaldehyde solution for 24 hours but is killed by peracetic acid in 30 seconds. Peracetic acid is a strong oxidizing agent leaving acetic acid as a residue. It is active both in an acid and neutral solution. The only limitations is its unstability in the presence of metals and oxidizable substances. This requires strict cleanliness and the protection of most metal parts with enamel or plastic.

While peracetic acid even in a 1% solution has an irritating, pungent odor, it has no germicidal properties in the gaseous state. Hence it is necessary to wet the entire surface of the suit or protective garment together with the complete interior of the entry lock. The germicidal spray must also sweep all contamination from the air. In connection with the plastic dry boxes we have found that a stainless steel pneumatic atomizing nozzle used with sterile compressed air at 25 lbs. gauge pressure will sterilize the entire container and attached gloves from one position. The gloves were moved around during the spraying. This demonstrates the feasibility of sterilizing a man in a protective garment within an entry lock. It is planned to use several pneumatic nozzles to ensure adequate distribution of the spray.

Incidental to this problem, it was found that the plastic dry boxes are excellent for rearing sterile plants and culturing microorganisms. Both a dip bath and a spray sterilized entry lock have been used successfully. The plastic containers are fragile even though they are made from 20 mil vinyl film so their place in the germfree laboratory is yet to be determined.

Two types of entry locks to a sterile tank or room have been devised and preliminary models tested on the mock up tank in the Biology Building. The first consists of two liquid sealed hatches covering the entry lock. These are made from a light stainless steel lid 23 inches square with a 6 inch skirt attached to the rim. The skirt fits into a trough containing the sealing liquid and surrounding the entrance hatch. This device is merely an inexpensive air tight door. Any other type could serve as well. The second consists of a cylindrical chamber formed of flexible plastic attached to a door jam and resembles a shower curtain. The bottom of the curtain is either weighted or attached to a plastic basin which serves as a germicidal trap and seal. The curtain is split on opposite sides by a gas tight plastic zipper

which enables the operator to enter the lock, sterilize the lock and garment, andthen enter the sterile room. When the zipper is closed the fastener is submerged in the germicide in the basin.

This arrangement eliminates submerging in liquid and makes it possible to exhaust the air of the garment through a sterile filter thus doing away with the cumbersome exhaust hose. The germicide can be readily washed from the suit with sterile water or neutralizer before the worker enters the sterile room. Since the garment will not be subjected to the strains encountered during submerging, it can be designed for comfort by the use of rigid structures to increase the freedom of movement. Thus it seems possible to design a completely sterile room or laboratory in which research personnel work in sterile protective garments. This procedure may remove many of the space and manipulation limitations of the present germfree apparatus.

III. BIOCHEMISTRY AND NUTRITION

FROM: T. D. Luckey, Chief Biochemist
(With the assistance of M. Beaver, L. MacAllister, T. Mende, A. Pappas, J. Pleasants,* L. Takacs,** In collaboration with M. Forbes (University of Pennsylvania) and E. Hawk (NIH) who are in residence at LOBUND Institute.)

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report, 1 January 1953 - 30 June 1953

DATE: 31 July 1953

A. Biochemistry:

1. Survey of the Germfree Chick:

Previous reports have indicated the gross composition of the liver and cecal contents of the germfree White Wyandotte Bantam was similar to that of conventional control birds. (One exception to this was the relatively large amount of protein found in the cecal contents of conventional birds.) Niacin and pantothenate were higher in germfree birds while pyridoxine and biotin were low in germfree cecal contents. More detailed study was presented of the vitamin interaction in White Leghorn chicks showing how vitamin metabolism is effected by specific vitamin deficiencies and giving the significance of statistical studies on composition of the main organs.

Further study along this line indicates the relative "B-vitamins priority" of different organs when compared to the diet or the total chick (Figure 1).

In general it is noted that the B-vitamin content of the diet is grossly comparable to that of the blood and total chick. Exceptions to this in the blood are low values found for riboflavin and possibly niacin (at least niacin is low in the blood of conventional birds); while the folic acid content is much lower (possibly due to the level of citrovorum factor - not measured). The brain muscle and cecal contents generally carry higher vitamin contents than those found in the diet or the total chick. And the liver generally contains a concentration of B-vitamins 20-50 times higher than the blood or diet. This selective retention by certain tissues of physiologically active compounds presents an interesting problem: How can the liver cells take a compound against a 50X concentration gradient?

Another interesting feature is the fact that the germfree birds excrete as large quantities of B-vitamins (as measured in the ceCum) as do conventional birds. On this basis the excreta contain higher concentrations of B-vitamins than the food.

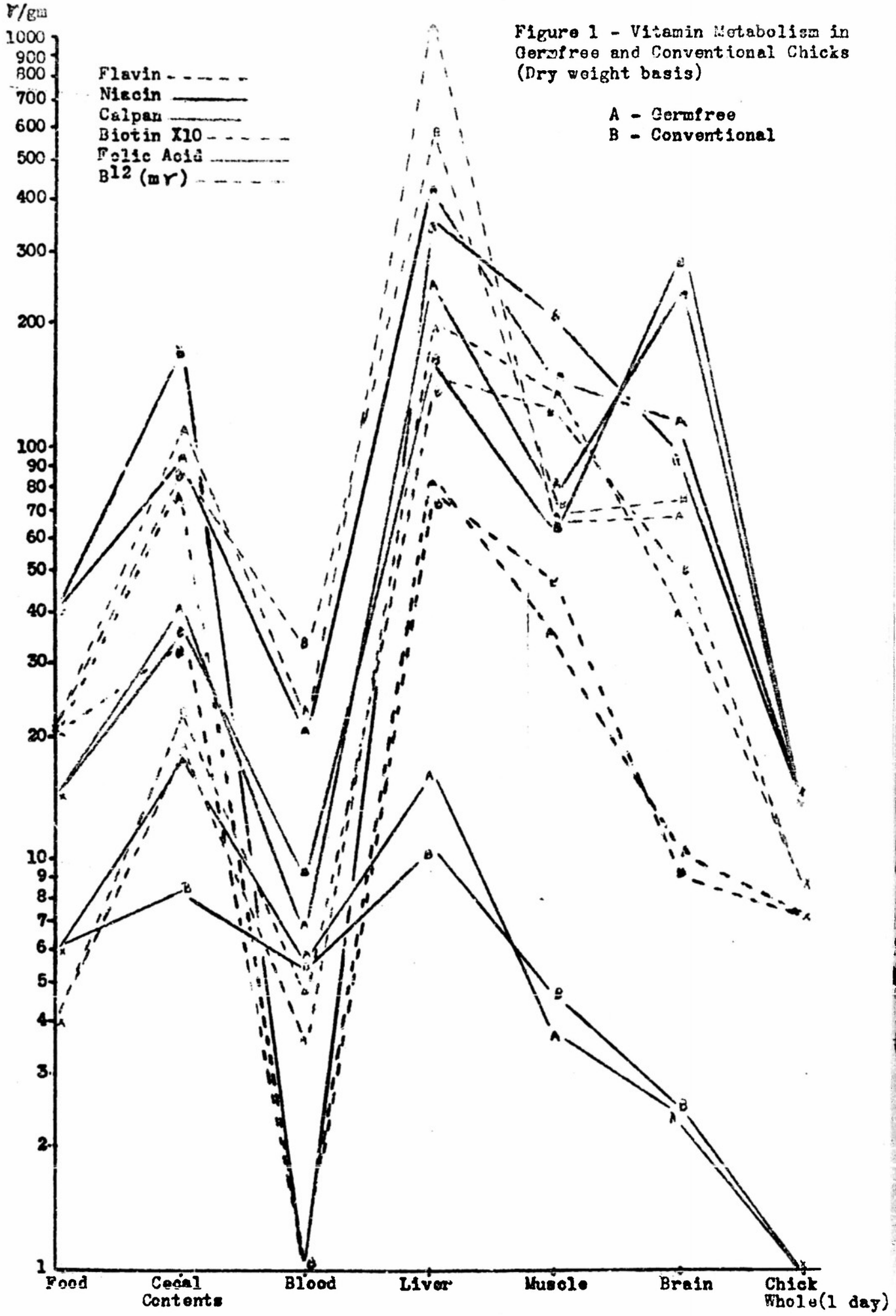


Figure 1 - Vitamin Metabolism in Germfree and Conventional Chicks (Dry weight basis)

A - Germfree
B - Conventional

2. Biochemical Survey of Germfree Rats:

Data were taken from germfree rats killed in November 1951 and conventional rats killed by overheating to give a more close control. The results are summarized in Table I with rats of the age categories 66, 162, and 256 days.

No great differences were found in the gross chemical composition of the tissues of the two groups of rats. Small differences or possible differences are mentioned as possible significant differences. The livers of the germfree and conventional rats were very similar in dry weight, fat, nitrogen and ash content. The older conventional rats showed a rise in liver ash not seen in the older germfree rats.

The cecal contents of germfree rats appears to contain more water than is found in conventional rats. And the ash content of the cecal contents is lower on a dry weight basis.

The dry weight of the brain of germfree rats may be slightly greater than that of conventional controls. The ash and fat content of the brain appear to be similar.

The dry weight of the muscle tissue also is somewhat greater. It is possible that the larger period of heat treatment for germfree rats (2-6 hrs. after death) affected the dry weight values of the brain and muscle. The ash, fat and nitrogen values appear to be very similar.

Kidney tissue was similar in dry weight ash (except some of the old conventional rats had increased ash content), fat and nitrogen content.

Bone ash and fat values were the same for the two groups. The phosphorus content of the bones was again very similar.

The dry weight of the spleen of germfree rats may be somewhat less than that of conventional rats.

Statistical analysis should clarify these minor points. The main point is that heat killed germfree and conventional rats were very similar in biochemical composition of their tissues.

3. Analysis of Rat's Milk:

A summary of the composition of rat's milk is given in Table II with the data obtained at LOBUND Institute compared with that of other investigators. The fat value is presented as being lower than that given for other investigators probably because they used milk near the end of lactation. At this period our values agree well with those of other workers. However, the low value obtained from 6-16 days brings the average down. A similar case may be stated for the ash values.

TABLE I

BIOCHEMISTRY OF RATS - GERM FREE (A) COMPARED TO CONVENTIONAL (B)

LIVER

	AGE: 66 days				162 days			265 days			All
		N	Ave.	Range	N	Ave.	Range	N	Ave.	Range	Ave.
Dry wgt.	A	4	29.0	27.8/30.2	4	29.4	27.9/31.5	5	30.5	29.0/328	29.7
	B	5	29.0	27.5/29.2	5	28 .4	27.5/29.3	3	29.0	29.0/29.1	28.4
Ash	A	5	5.01	4.44/5.39	4	4.77	4.51/5.04	5	4.81	4.46/5.30	4.87
	B	5	4.84	4.89/5.18	4	4.25	4.00/4.69	3	6.16	5.73/6.56	5.11
Fat	A	5	6.80	5.69/7.36	4	6.76	6.32/7.70	4	9.89	8.82/11.2	7.74
	B	5	6.43	5.23/7.17	3	6.93	6.6/7.3	3	6.61	6.60/6.90	6.65
N_2	A	5	3.52	2.61/4.50	4	3.72	3.26/3.76	5	3.07	2.85/3.72	3.37
	B	5	2.51	2.21/2.73	5	3.34	3.13/3.54	3	3.03	2.71/4.66	3.03
CECAL CONTENTS											
Dry wgt.	A	5	16.9	15.0/19.2	2	25.1	21.3/28.9	5	16.8	15.3/19.0	18.2
	B	4	22.5	21.5/23.6	5	27.9	26.3/28.9	3	24.8	24.5/25.1	25.3
Ash	A	4	14.4	12.9/16.6	2	15.9	15.8/15.9	5	15.9	14.6/17.6	15.3
	B	4	21.1	19.5/23.3	4	23.2	21.6/25.0	3	24.0	23.5/24.3	22.6
BRAIN											
Dry wgt.	A	3	20.7	20.1/21.6	3	21.7	20.1/23.4	5	20.9	19.5/21.5	21.1
	B	5	19.3	17.2/20.8	5	19.2	16.2/21.8	3	20.3	18.3/21.3	19.5
Ash	A	4	7.28	6.82/8.80	3	6.98	6.69/7.56	4	7.55	7.06/8.14	7.50
	B	5	6.61	6.19/7.10	5	7.61	6.31/8.89	3	8.97	8.00/10.10	7.54
Fat	A	3	9.30	8.23/11.0	3	8.54	4.41/12.5	3	11.5	9.50/12.20	9.78
	B	5	8.79	8.26/9.70	3	9.93	9.30/10.60	3	9.93	9.30/10.60	9.34
N_2	A	-	-	-	-	-	-	-	-	-	-
	B	4	1.78	1.73/1.95	5	1.65	1.44/1.85	3	1.64	1.42/1.83	1.69

MUSCLE

		AGE: 66 days			162 days			256 days			All
		N.	Ave.	Range	N.	Ave.	Range	N	Ave.	Range	Ave.
Dry wgt.	A	4	28.0	26.3/29.2	4	26.3	25.3/27.5	5	28.2	26.2/29.6	27.5
	B	5	25.0	24.6/25.7	4	24.5	23.5/25.5	3	25.7	24.5/26.5	25.0
Ash	A	5	6.00	4.97/7.42	4	4.75	4.63/4.92	4	5.56	5.40/6.06	5.51
	B	4	4.58	4.35/4.93	4	5.73	4.36/6.93	3	5.85	5.17/6.26	5.35
Fat	A	5	2.74	2.91/3.67	3	3.95	3.61/4.13	5	4.45	3.62/5.32	3.68
	B	4	4.00	3.72/4.33	3	3.40	3.30/3.50	3	3.40	3.30/3.50	3.70
N_2	A	4	3.74	3.46/4.00	4	4.06	3.97/4.20	3	3.65	3.07/3.96	3.90
	B	5	2.90	2.50/3.15	5	3.39	3.26/3.52	3	3.29	3.27/3.30	3.18
KIDNEY											
Dry wgt.	A	4	24.9	22.9/27.7	4	25.1	23.9/27.3	5	25.8	25.8/26.9	25.3
	B	5	25.1	22.9/27.7	5	25.5	23.9/27.3	3	26.2	25.8/26.9	25.5
Ash	A	4	6.22	4.86/7.80	4	7.15	4.70/10.10	4	5.97	4.54/7.70	6.42
	B	5	6.08	5.21/7.25	5	7.07	5.10/10.10	3	8.91	6.10/12.10	7.12
Fat	A	4	5.74	5.06/6.02	4	4.93	4.48/5.44	5	6.00	5.44/7.17	5.59
	B	4	5.48	5.26/6.00	3	6.70	6.40/6.90	3	6.70	6.40/6.90	6.10
N_2	A	5	2.90	2.41/3.45	4	2.52	2.33/2.72	4	2.98	2.82/3.32	2.81
	B	5	2.52	2.06/2.77	5	2.77	2.60/3.07	3	2.49	2.39/2.60	2.61
BONE											
Dry wgt.	A	-	-	-	-	-	-	-	-	-	-
	B	5	62.3	60.2/64.9	5	75.9	71.7/78.8	3	70.9	69.4/72.2	69.5
Ash	A	5	64.5	60.8/67.0	4	68.5	68.4/68.6	5	71.0	70.5/71.6	68.0
	B	5	65.3	65.0/56.7	5	69.6	68.8/70.6	3	69.6	69.4/69.8	68.0
Fat	A	3	1.9	1.8/1.9	3	3.5	3.3/3.7	3	3.4	3.4/3.5	2.9
	B	-	-	-	3	2.0	1.8/2.0	3	2.9	1.7/4.6	2.5
P, mg%	A	3	209	197/217	4	205	194/217	5	199	178/219	203
	B	4	195	191/203	5	192	189/195	2	184	180/187	191
SPLEEN											
Dry wgt.	A	3	23.7	21.9/25.3	4	24.4	23.5/25.4	4	25.0	23.3/25.8	24.3
	B	4	25.1	24.5/25.6	-	-	-	3	27.2	23.6/29.7	26.0
Ash	A	5	7.67	6.84/7.64	3	6.54	6.40/6.74	5	6.66	6.24/7.16	7.02
	B	4	5.79	5.50/6.59	-	-	-	3	9.23	6.28/11.0	7.37

Table II

Comparison with Literature Data

Component	LOBUND(1)	Cox-Mueller	Mayer (2)	Houston & Kon
Dry wgt. %	29(3)	31.7	22.2	27.9
CHO %	3.74	2.83	3.39	
Fat %	10(3)	14.79	12.4	13.8
Protein %	9.2	11.77	6.89	
Casein %	7.5	9.20		
Globulin %	1.01	-		
Albumin %	0.59	0.91		
NPN %	0.082	0.21	0.438	
Ash %	1.2(3)	1.50		
Ca %	0.234	0.349		
P %	0.233	0.272		
K %	0.119	0.170		
Na %	0.140	0.076		
Fe %		0.0007		
Mg %		0.031		
Cu %		0.0007		
Cl %		0.117		
Vit. E mg%				
Vit. A γ/gm				9.2
Vit. C mg%	1.2			0.35
Thiamin γ/gm				1.44
Flavin γ/gm	2.64			6.07
Niacin γ/gm	17.5			
Pantothenate γ/gm	5.5			
Pyridoxine γ/gm	0.76			
Biotin γ/gm	0.082			
Folic γ/gm	0.33			
Vit. B12 γ/gm				
Carotene	0.0			
pH	6.7	6.6		
Sp. G.	1.0372	1.047		
Fat globule size μ	4.0			
Osmotic pressure (≅H_2Cl)	0.160			
Viscosity	6.4			
Surface Tension (dynes/cm^2)	49.6			
Max. value (ml/day)	18.0	8.0		6.5

(1) Exclusive of the first 5 days lactation.
(2) Stomach contents
(3) Average value for ash, dry weight and fat are not necessarily representative as shown by curves presented previously.

B. Nutrition:

1. Diet Laboratory:

As indicated in the last report, there appears to be an ever increasing demand for the services of the diet laboratory. The total food has increased during this six months at the same rate it increased during the previous six months (see page 12, ONR Report December 1952). During the past month the load has eased after a concented effort by everyone to use no more diet than absolutely essential. A work analysis of the two girls in the diet kitchen indicates a helper could be used since 1/4 of their time is spent doing routine jobs.

One innovation which is worthy of mention is the use of polyethylene bags for the storage of diets. The advantages are; ease of storing because they pack well, the labeled edge is readily seen in an ice chest, exact amounts given since each is made to 1 Kg, and no chance of contamination from a previous diet since each is used only once for critical diets.

2. Chicken Nutrition Studies:

The projects using germfree chicks have been almost completely inactive due to successive contamination at hatch during the early months (see Sections II and IV) and the cages were assigned to the virus program through June.

Testing of the merry-go-round cage was completed (for conventional chicks). This cage is designed to make six groups of chicks equivalent environmentally. Briefly described, it is a rotating (one revolution per 3 minutes) pie with six triangular cages for chicks. In the first test the chicks in this unit grew about 7% faster than clutchmates in a commercial battery. However, this increased growth rate was not seen in subsequent tests.

3. Rat Nutrition Study:

Two germfree rats have been on a "vitamin balance" study for several months. The experiment is completed and analyses of tissues for vitamins is almost completed. A more complete report will be given later.

C. Rearing a New Germfree Species:

During our work on the action of antibiotics in germfree chicks it became very evident that turkeys were the bird of choice for this problem. Consequently, turkey eggs were treated in a manner very similar to the technique used to obtain germfree chicks, set and hatched in the germfree cage. The turkey poults were germfree and experiments were run in the same cages which had been used for chicks. The hatch was generally acceptable, the growth and general appearance were good. The poults grow so fast that they fill the available space by six weeks of age. Hence, they are not desired for longer experiments. Details will be presented in a separate publication in the near future.

D. Collaborative Programs:

1. A Study of the Etiology of Hemorrhagic Liver Necrosis with Dr. Paul Gyorgy and Dr. M. Forbes (in residence) from the University of Pennsylvania Medical School:

Details of this project were presented to the New York Academy of Sciences, 14 February 1953 and will appear in a separate publication in the proceedings of that Society. In summary of the work to that date we stated: "In a series of four experiments, eight germfree rats were found to have normal livers at autopsy; all but one of the 34 conventional rats, fed the same diet, died with massive hemorrhagic necrosis of the liver. The germfree rats exhibited prolonged clotting time which was partially prevented by vitamin E supplementation". It was noted in the paper that the germfree rats ate more and grew better than the conventional rats. In the fifth experiment the food intake of the germfree rats was restricted and five of the six germfree rats died with necrotic livers. Thus living bacteria are not necessarily involved directly in this syndrome.

2. The Role of Ascorbic Acid or Antibiotics in Replacing the Rat's Requirement for Certain B-Vitamins. Collaborative Study with Drs. F. S. Daft and E. A. Hawk (in residence) of the Institute for Arthritic and Metabolic Diseases, U. S. Department of Health, Education and Welfare:

Work in the past 6 months was occupied with the development of suitable diets which could be sterilized and which would demonstrate the effect of replacement of certain B-vitamins with ascorbic acid or antibiotics. Thus far, steam appears to be superior to ethylene oxide for diet sterilization. Cathode ray sterilization appears to be preferred for labile material such as vitamin C.

Work with riboflavin appears to be more practical than with pantothenic acid under the special conditions at LOBUND Institute.

The preliminary experiments have progressed far enough to indicate that a critical experiment using germfree rats is feasible, whenever rats and equipment are available.

E. Future Developments:

The biochemistry division will continue to act as a service laboratory in the future as it has in the past. The research will continue as animals are available. The development of rearing new species becomes more of a service to Mr. Pleasants in the new setup.

The projected hopes and needed equipment mentioned in past reports remain important.

It is hoped that replacement of personnel lost from this division during the past six months will be made up during the next six months.

IV. BACTERIOLOGY AND SEROLOGY

FROM: M. Wagner, Chief Bacteriologist, (with the assistance of J. Dingler, A. DeLeva, B. McClain, D. Moran and M. Osterhout)

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report 1 January 1953 to 30 June 1953

DATE: 31 July 1953

A. Bacteriological Testing of Germfree-type Cages:

This function of the Bacteriology Laboratory continues as a service to various LOBUND projects being run in germfree-type apparatus.

Special mention is made regarding the large number of Pseudomonas contaminations experienced in the production of germfree chickens during the January-June 1953 period.

Chicken Experiments from 1 January - 30 June 1953

	Germicidal Treatment of Eggs	
	Mercuric Chloride(1)	Detergent-Sanitizer(2) (Rohm & Haas)
Experiments attempted	25	6
Experiments germfree (total)	4 (16%)	5 (83.3%)
Experiments contaminated(total)	21 (84%)	1 (16.7%)
Pseudomonas	18 (85.7%)(3)	0
Other than Pseudomonas	3 (14.3%)(3)	1

Footnotes to Table

(1) Standard treatment at 0 and 20 days incubation with 2% $HgCl_2$ at 38° C for 5 minutes.

(2) Eggs are dipped in 0.25% "Detergent Sanitizer No. 115" (Rohm & Haas) for 3 minutes at 0 days, 5 minutes at 20 days incubation. No brushing or rinsing is used. The active germicide is alkyl (C_9 to C_{15}) tolyl methyl trimethyl ammonium chloride incorporated as 5% of the powdered product and used at approximately 0.015% in final solution.

(3) Calculated as % of total contaminations.

The table shows contamination during this period in 84% of experiments attempted with $HgCl_2$ treated eggs and only 16% germfree. Pseudomonas was isolated in 85.7% of the total contaminations encountered. This incidence of contamination is extremely high when compared to previous series of chicken experiments in which the expected percentage of germfree experiments for $HgCl_2$ treated eggs ranged between 70-90% of the experiments attempted.

Because of the repeated contaminations with Pseudomonas, Detergent Sanitizer No. 115 was substituted for $HgCl_2$ in treating the eggs. This product was specifically developed for egg washing and has been described by Rohm and Haas Co. as being particularly active against pseudomonads causing "green rot" in eggs. The table shows that in six experiments attempted with "Detergent Sanitizer No. 115", five resulted in germfree hatches. The sixth experiment resulted in a Micrococcus contamination probably due to a cut diaphragm in one of the valves on the cage. No Pseudomonas was detected in this latter case. Finally it should be noted that the first group of "Detergent Sanitizer No. 115" treated eggs resulted in a germfree hatch followed by four groups of $HgCl_2$ treated eggs, all of which showed Pseudomonas contamination. The next 5 hatches were from Detergent Sanitizer treated eggs and all were germfree except for the one accidental contamination. While no comparative experiments could be run on the same batch of eggs using the two different treatments (because of limited equipment) the indications, as given above, are that the "Detergent Sanitizer No. 115" has eliminated the Pseudomonas contamination problem.

"Detergent Sanitizer No. 115" is now being used routinely for treatment of all eggs to be used in germfree chicken production.

B. Sterilization of Diets and Dietary Ingredients:

Most of the diets used for germfree animal experimentation are sterilized into germfree type apparatus via autoclaving procedures. Generally, partial destruction of the more labile dietary constituents by steam sterilization can be compensated for by incorporation of higher levels of the labile components in the diet. However, in certain types of experiments steam sterilization may produce objectionable reactions such as carmelization of high sugar diets, dextrinization of starch diets, excessive destruction of heat labile substances, etc. which may render the diet unsuitable for the demands of the experiment. Under such conditions, other methods of sterilization must be attempted. At LOBUND, we have tried sterilization of sucrose, vitamin C and antibiotics as well as complete diet by one or more of the following treatments:

1. Ethylene Oxide gas
2. Alcohol - heat treatment
3. Soft x-ray irradiation
4. Cathode electron radiation

All materials to be treated were packaged in poly-ethylene bags(1). Samples were inoculated with a concentrated spore suspension of Bacillus globigii or Bacillus stearothermophilus in ratio of 1 ml per 100 gms diet. Poly-ethylene or glass packaging was necessary in order to protect the sterilized material since passage into the germfree-type apparatus is accomplished via a germicidal trap. In several of the ethylene oxide gas treatment experiments, cloth wrapped samples were run

(1) Glass ampules were used for packaging sucrose to be used in alcohol-heat treatment.

in parallel with the polyethylene packaged samples in order to check the possibility of failure of the gas to penetrate the polyethylene plastic.

1. Ethylene Oxide Gas Treatment (with assistance of R. Hoover & Dr. E. Hawk)

Run I: Diet 358 inoculated with Bacillus globigii spores

Package	Pressure EO P.S.I	Time	Temp. Variation	Recover of B. globigii
Triple Polyethylene Bags	High 23 lbs. (fluctuated with temp.)	6hrs	33 - 63°C	Positive

Run II: Diet 358; Sucrose; Vitamin C; inoculated with B. globigii spores

Package	Pressure EO P.S.I.	Time	Temp. Variation	Recovery of B. globigii
Triple Polyethylene Bags	High 26 (fluctuated with temp).	6 hrs	30 - 60°C	Positive

Run III: Diet 358; sucrose; inoculated with B. globigi

Packaging	Pressure EO P.S.I.	Time	Temp.	Recovery of B. globigii
Cloth	3	1 hr .	Room temp.	Positive
Single polyethylene	"	1 hr.	" "	"
Triple polyethylene	"	1 hr	" "	"

Run IV: Diet 375; sucrose

Packaging	Pressure EO P.S.I.	Time	Temp.	Recovery of B. globigii
Cloth	6	24 hrs.	Room	Positive
Cloth	35	"	60° C	"
Single polyethylene	6	"	Room	"
Single polyethylene	35	"	60° C	"
Triple polyethylene	6	"	Room	"
Triple polyethylene	35	"	60° C	"

Ethylene oxide used at pressures as high as 35 lbs/sq in. at temperatures up to 60° C and for periods of time up to 24 hours failed to completely destroy Bacillus globigii spores inoculated into whole diet or sucrose. The treatment was ineffective in cloth packaged samples as well as in the polyethylene bags.

2. Alcohol-Heat Treatment (with assistance of Dr. Hawk)

Autoclaved gramular sucrose or high sucrose containing diets are unsuitable for germfree experiments because of the high degree of carmelization which takes place. While aqueous solutions of sucrose can be autoclaved, the recovery of the dry sterile sucrose (by evaporation of the water) for mixing with other autoclavable dietary ingredients inside a germfree cage presents a difficult problem. Dr. Hawk of NIH (presently stationed at LOBUND) suggested the possibility of using a combined alcohol-heat treatment for sterilization of sucrose inside a sealed glass container. Preliminary tests showed:

a) Sucrose in ethyl alcohol is not carmelized by autoclaving.

b) Sucrose is not very soluble in 95% alcohol, leaving the majority of the sucrose in the undissolved granular form. This feature is important from the standpoint of recovery of the dry sucrose after autoclaving. Most of the alcohol can be decanted and the residual alcohol more readily evaporated than water.

Twenty gram samples of sucrose were inoculated respectively with 0.2 cc of concentrated spore suspensions of Bacillus globigii or Bacillus stearothermophilus, layered with 20 ml of 95% ethyl alcohol, sealed into 50 ml capacity glass ampules and autoclaved. A sample of sucrose-in-water was run in parallel.

Sample	Spore Inoculum	Autoclave Pressure (lbs./sq.in.)	Time hrs.	Recovery of Inoculum
Sucrose-alcohol	B.globigii	15	0.5	+
"	"	20	1	+
"	B. stearo.	15	0.5	+
"	"	20	1	+
Sucrose-H_2O	B. stearo	20	1	+
"	"	Untreated	0	+

Autoclaving under 20 lbs. steam/sq. in. for 1 hour failed to destroy spores of Bacillus globigii and Bacillus stearothermophilus in a sucrose-alcohol (95%) system while B. stearothermophilus was destroyed in a sucrose-H_2O system. Trials were not made with diluted alcohol since the increased solubility of sucrose with additional hydration defeated the original purpose of the method.

3. X-Ray Irradiation (with assistance of Dr. Hawk and Mr. W. Scrugga)

Attempts were made to sterilize samples of Vitamin C seeded with B. globigii or B. stearothermophilus spores by X-ray irradiation. The

source of X-ray was from a Picker Deep Therapy 260 KVP X-ray machine with no added filtration. The dose delivered was 1,500,000 r to samples of spore seeded Vitamin C packaged in (1) single polyethylene bag; (2) tin foil; (3) polyethylene + tin. Tin was used to provide a fluorescent X-radiation.

Packaging	Spore Inoculum	Recovery of Inoculum
Polyethylene	B. globigii	-
Tin	"	+
Poly + Tin	"	-
Untreated	"	+
Poly	B. stearo.	+
Tin	"	+
Poly + Tin	"	+
Untreated	"	+

X-ray treatment failed to destroy the Bacillus stearothermophilus spores seeded in Vitamin C. The results with B. globigii are questionable since one might expect the "polyethylene + tin package" to be positive if the survival in the tin-only wrapped package is correct.

4. Cathode Electron Treatment (with assistance of Dr. Luckey)

Cathode ray treatment (1) has been used for sterilization of antibiotics to be used in germfree experimentation, particularly in the "Studies on the Growth Effect of Antibiotics in Germfree Animals".(2) Reference to successful sterilization of unseeded samples of bacitracin, procaine penicillin and terramycin is made in that fowl fed electron treated antibiotics remained germfree in 8 chicken and 2 turkey experiments(2). Unseeded samples of crystalline Vitamin C have also tested sterile after cathode ray treatment.

More recently, a more severe test for the method was provided by a complete diet (L-109) which was seeded with Bacillus globigii spores, packaged in triple polyethylene bags and submitted to cathode radiation at a minimum of 2 million rep intensity.(3). The sample returned to LOBUND tested sterile.

1. Electronized Chemical Corp., Brooklyn, N. Y. Treatment with cathode ray at 1.5 million rep intensity.
2. Special Report to ONR, June 4, 1952.
3. High Voltage Engineering Corp., Cambridge, Massachusetts.

At present we have submitted to High Voltage Engineering Corp. a 500 gram sample of diet seeded with the highly resistant Bacillus stearothermophilus spores for similar treatment. We are awaiting return of the material for test.

In our experience, only one incidence of contamination of electronically treated material has been encountered. A yeast-like organism was isolated from two of four samples of streptomycin tested. These individual samples were all from the same batch of streptomycin and were packaged and treated at the same time.

Barring poor results from the B. stearothermophilus seeded diet mentioned above, the cathode electron treatment appears to offer the greatest promise for sterilization of non-autoclavable materials. The greatest disadvantage to the method is the non-availability of the process in this area and the delays encountered in getting materials processed in quantity.

C. Dental Caries Project (Collaborative Project between LOBUND Institute and the Zoller Memorial Clinic, University of Chicago).

1. Cariogenesis in Rats with Lactobacillus Monoflora.

In the July 1, 1952 - December 31, 1952 report to ONR, gross observations on rat molars from experiment indexed 39D1-9 were reported. Microscopic examination of the rat molars failed to reveal any carious lesions in Group A (germfree). Group AI (Lactobacillus #465 monoflora) was reported as having no gross lesions. However, upon microscopic examination, one of 4 rats in this group showed one possible enamel lesion. The dentine was not involved.

Group AIB (littermate controls to the rats in the AI monoflora group) and Group B (diet controls) were reared on the cariogenic diet under conventional laboratory conditions and all showed characteristic type lesions.

2. Cariogenesis in Rats with Streptococcus liquefaciens Monoflora.

Experiment 39D2-1 was briefly described in the previous report as being underway. The design of this experiment was similar to the Lactobacillus monoflora experiment 39D1-9 above except that Streptococcus liquefaciens #539 monoflora was substituted for the lactobacilli. This experiment has terminated as of June 1, 1953 and the heads sent to the Zoller Memorial Clinic for caries evaluation. In the latest communication from Dr. F. J. Orland, he states that 3 of the four rats in the enterococcus inoculated group show molar fractures and some peculiar lesions in central sulci as if the process had been arrested (gross examination only). The control rats almost all showed lesions of the usual type expected. Final results of the microscopic and macroscopic caries evaluation will be included in the next report.

3. Cariogenesis in Rats with Acidogenic and Proteolytic Diflora.

Currently, we have underway experiment 39D3-1. This experiment is again patterned after those mentioned above. However, the experimental group (AI) has been purposely inoculated orally with two strains of organisms isolated from a conventional rat carious lesion:

(1) An acidogenic coccus characterized as a strain of Streptococcus fecalis.

(2) A proteolytic Gram positive, non spore producing rod (unidentified). The termination date is scheduled for November 1953 and data should be available for the next report.

Two caries experiments are currently underway involving conventional laboratory rats only.

4. Cariogenesis in Quonset Colony Rats.

Experiment 25J2-1 involves cariogenesis in conventional rats maintained in the Quonset Animal Colony at LOBUND on a cariogenic diet (L-128 + 5% dextrose water). Previous experience has shown that whereas rats maintained on a cariogenic diet in the Biology Building animal room produced a high incidence of dental caries; rats maintained in the Quonset colony (during the period of close isolation between November 6, 1950 and February 6, 1952) showed very low incidence. Now that strict isolation procedures are no longer followed in the Quonset colony, it is desireable to determine what the cariogenic incidence will be. The answer to this question will determine the advisability of maintaining conventional control animals in the Quonset colony for future experiments in the caries projects since we eventually contemplate discontinuation of the Biology Building animal room.

5. Effect of Abietic Acid on Cariogenesis.

Experiment 25J2-2 involves cariogenesis in conventional rats fed cariogenic diet L-128 to which 1% abietic acid derivative has been added (new diet No. L-386) and 5% dextrose water fed ad lib.

In the semi-annual report to ONR dated August 15, 1950, data was presented to show that rats fed a cariogenic diet and housed on wire screen bottom cages showed 100% (16 of 16 rats) caries incidence. However, rats fed the same dietary regimen showed only 40% incidence (4 of 10 rats) when maintained on pine wood shaving bedding instead of wire screen. Similarly with pelleted cariogenic diet, 100% caries incidence (7 of 7 rats) was experienced with rats on wire screen but only 20% (2 of 10 rats) in rats kept on wood shavings.

In the next report submitted December 31, 1950 (p. 13 & 14) it was reported that 1% wood shavings added to a glucose medium markedly inhibited acid production by Lactobacillus #465.

In the present experiment 1% abietic acid derivative is being fed with the cariogenic diet to test the role of abietic acid as the active substance in pine wood shavings responsible for lowered caries incidence.

6. Bacterial Counts from the Oral Cavity of Rats on the Dental Caries Project 39D2-1.

Dental caries project 39D2-1 involved the cariogenesis produced by the presence of Streptococcus liquefaciens as a monoflora in rats maintained in germfree-type apparatus. Quantitative bacterial counts were run on samples taken from swabbing of the oral cavity of the mono-contaminated AI group of rats as well as the two control groups AIB and B. Total counts were run on brain heart infusion + 5% horse serum agar. Streptococcus liquefaciens counts were run on Difco-Azide Blood Agar Base agar to which 0.5% salicin and 0.4% gelatin were added. Salicin fermenting colonies were identified by flooding the incubated plates with brom cresol purple and counting the yellow zoned colonies. The plate was then flooded with acid $HgCl_2$ reagent and gelatin proteolysis recorded by counting colonies surrounded by a clear zone. Salicin positive, gelatin positive streptococci were recorded as Streptococcus liquefaciens. It is emphasized that in the AI (monoflora) group, the total count and streptococcus count are identical.

Bacteria per Gram Oral Sample

Bacteria	Group	No. Rats.	No. Cultures	Minimum	Maximum	Average
Total Count	AI	4	26	5.44×10^5	2.42×10^8	4.20×10^7
	AIB	3	21	3.04×10^7	1.60×10^9	4.54×10^8
	B	4	24	2.13×10^6	1.14×10^9	2.43×10^8
Streptococcus liquefaciens	AI	4	26	5.44×10^5	2.42×10^8	4.20×10^7
	AIB	3	18	0	9.40×10^6	2.11×10^6
	B	4	20	0	8.85×10^5	1.04×10^5

The figures for the Streptococcus liquefaciens count of the AI group in the above table is not quite accurate in terms of the following findings:

At the start of the experiment, all colonies isolated on the azide-salicin-gelatin plates prepared from the AI group of rats showed a positive salicin-positive gelatin reaction. However, in later cultures it was found that in addition to the above mentioned colonies, salicin positive - gelatin negative colonies also started to appear on some of the plates. Further study of the gelatin negative strains showed them to be typically acting strains of Streptococcus fecalis. Indeed, Bergey's Manual (6th edition) pg. 327 states that gelatin liquefaction fails in occasional variants of S. liquefaciens; Sherman, Stark and Maurer (J. Bact. 1937 Vol. 33) state that S. fecalis and S. liquefaciens differ only in proteolytic activity. We therefore are inclined to look at the appearance of the gelatin negative strains, not as a contamination of Streptococcus fecalis from an outside source but rather a mutation or

variation through which loss of the proteolytic character has occurred. Again Sherman, Stark and Maurer state that loss of characteristics (whether by mutation or gradual changes) are not uncommon.

It is also interesting to note that the above-mentioned loss in proteolytic activity was only noted in cultures taken from the rats. Efforts to demonstrate non-proteolytic colonies in platings from the original test tube culture (or subcultures) used to inoculate the rats have failed in seven attempts. It may be that growth in the rat or in the cage environment may favor such changes.

It is improbable that similar changes can be checked in the AIB or B groups since Streptococcus fecalis and S. liquefaciens occur in the intestinal tract of conventional rats. The problem of whether these were established as independent entities or whether one exists as a continually occurring variation from the other would be difficult to resolve.

The following table records the occurrence of the gelatin + and - strains in the rats comprising the AI group. The proteolytic strain predominated throughout.

Incidence of Gelatin positive and Gelatin negative Enterococci in Group AI Rats (expressed in millions per gram oral sample)

Culture Period	Rat No. 1			Rat No. 2			Rat No. 3			Rat No. 4		
	Total Count	S. liq.	S. fec.	Total Count	S. liq.	S. fec.	Total Count	S. liq.	S. fec.	Total Count	S. liq.	S. fec.
1	26.4	26.4	0	1.19	1.19	0	74.2	74.2	0	104.0	104.0	0
2	18.9	*	*	0.54	*	*	7.9	*	*	114.0	*	*
3	56.8	*	*	242.0	*	*	11.0	*	*	19.6	*	*
4	-	-	-	11.9	11.9	0	16.5	8.55	7.65	-	-	-
5	3.27	3.14	0.14	36.7	36.7	0	5.2	4.6	0.59	121.0	116.0	5.5
6	4.18	3.89	0.29	25.0	25.0	0	3.82	3.37	0.45	4.63	4.37	0.25
7	0.69	0.67	0.02	1.62	1.61	0.01	12.2	10.9	1.3	148.0	148.0	0

* Denotes lack of observation due to varying temperature gradients within the bacteriological incubator. In this case, high temperature did not affect growth of the bacteria but interfered with proteolysis so that differentiation between S. fecalis and S. liquefaciens could not be made.

D. Serology:

1. Agglutinins in Rats Maintained in Association with Single Strains of Bacteria.

The work with monocontaminated rats in the dental caries work has afforded the opportunity to study the serologic response of these animals to the presence of the organism as a pure viable bacterial culture in the environment. We have determined agglutinin titers in these animals against antigens prepared from the homologous bacteria to which the animals were exposed. In each case, the rats labeled AI represent those animals maintained

as the mono-contaminated group while the AIB are littermates of the AI group but which were brought to the outside laboratory animal room environment as conventionally laboratory reared controls.

Group	(Index 39D1-9) Lactobacillus #465		(Index 39D2-1) Streptococcus Liquefaciens #539	
	Rat No.	Titer	Rat No.	Titer
Group A (germfree)	26	0	-	-
Group AI (monocontam.)	16	1:16	1	1:1024
	17	1:16	2	1:1024
	21	1:16	3	1:1024
AIB (littermate controls to A and AI groups)	23	0	5	1:32
	25	0	6	1:128
	-	-	7	1:64
B (diet controls not littermates to above groups)	1	0	13	1:16
	-	-	14	1:2
	-	-	15	1:32
	-	-	16	1:2
	-	-	17	1:16

The "lactobacillus animals" only showed reactions in the monoflora group and at the relatively low titer of 1:16. The rats in Group A were negative as might be expected since Group A ran germfree. Group B was also negative. This rat did not receive an oral inoculum of the specific lactobacillus nor was the organism ever isolated from this animal. Lactobacillus #465 is a human strain of lactobacillus (serological group F of Univ. of Chicago) and has not been demonstrated as part of the flora of conventional rats in our experience.

However, Group AIB did receive an oral inoculum of Lactobacillus #465 and as shown in the December 31, 1952 report to ONR (p. 17-18) the specific lactobacillus was isolated from animals in this group in 36 out of 44 cultures run. Nevertheless, these animals failed to show agglutinins to Lactobacillus #465.

In the "Streptococcus liquefaciens animals", reactions were stronger and occurred not only in the monoflora group but also in the littermate orally inoculated controls and non-littermate non-inoculated controls. (No completely germfree group was run in this experiment). The presence of agglutinins for Streptococcus liquefaciens in the serum of the AIB and B group rats is not surprising since this organism and the very closely related Streptococcus fecalis occur as part of the "natural" oral and intestinal flora of conventional laboratory rats. The relatively high titers in the monoflora AI group is interesting since rats are generally poor agglutinin producers even when given parenteral injections. In this case parenteral injections were not given and the relatively high titers (for rats) against S. liquefaciens were produced by "natural" (non-injected) means.

2. Antibody Production in Germfree and Conventional Chickens in Response To Parenterally Injected Antigens.

The incidence of certain so-called "natural" circulating antibodies which react with bacterial and mammalian antigens has been described previously for the germfree and conventional chicken (1). The present report deals with antibody production in the germfree and conventional chicken in response to intravenous injection of antigenic materials:

a. Salmonella pullorum bacterin
b. Bovine serum

The injection schedules were not designed for maximum antibody production but were limited in order to detect possible smaller differences between germfree and conventional groups.

Agglutinin Production in Germfree and Conventional Chickens after Intravenous Injection with Salmonella Pullorum Bacterin*

Chick No.	Status	Titer Tube No.	Dilution
211	Germfree	7	1:128
214	Germfree	8	1:256
218	Germfree	7	1:128
		Average 7.33	
656	Conventional	6	1:64
657	Conventional	7	1:128
661	Conventional	8	1:256
662	Conventional	6	1:64
		Average 6.75	

*0.5 ml on 3 alternate days (2 billion cells per ml.)
Bled 7 days after last injection.

(1) ONR Report #3, April 1, 1949

Precipitin Production in Germfree and Conventional Chickens after Intravenous Injection with Sterile Bovine Serum*

Chick No.	Status	Titer Tube No.	Bovine Antigen Dilution
213	Germfree	13	1:4096
215	Germfree	14	1:8192
216	Germfree	13	1:4096
		Average 13.33	
659	Conventional	13	1:4096
660	Conventional	13	1:4096
663	Conventional	12	1:2048
665	Conventional	13	1:4096
		Average 12.75	

*0.1 ml., 0.2, 0.4 ml. undiluted bovine serum on 3 alternate days. Bled 7 days after last injection.

Salmonella Pullorum Agglutinins and Bovine Serum Precipitins in Phenolized-Saline** Injected Germfree and Conventional Chickens.

Chick No.	Status	S. pullorum Agglutinins		Bovine Serum Precipitins	
		Titer Tube No.	Serum Dilution	Titer Tube No.	Antigen Dilution
212	Germfree	0	0	1 (trace)	1:1
217	Germfree	0	0	0	0
220	Germfree	0	0	0	0
658	Conventional	0	0	1 (trace)	1:1
664	Conventional	1(trace)	1:2	0	0
666	Conventional	0	0	0	0
667	Conventional	1 (trace)	1:2	0	0

** 0.5 ml phenolized saline on 3 alternate days (0.35% NaCl-0.5% phenol). Animals bled 7 days after last injection.

The Salmonella pullorum injected group produced low but definite titers in both germfree and conventional chickens while the saline treated birds showed no titers. No differences were observed between the germfree and conventional chickens.

The bovine serum injected chickens showed very comparable titers in both the germfree and conventional groups.

Some of the chickens in the saline injected groups showed partial reaction with the antigens used. These reactions occurred only in the lowest dilutions that can possibly be run for the agglutination and precipitin tests. These were only trace reactions when compared to the four plus reactions observed in sera from the specifically injected chickens.

V. PHYSIOLOGY AND PATHOLOGY

FROM: H. A. Gordon, Chief Physiologist (with the assistance of W. Scruggs and P. Wolfe).

TO: J. A. Reyniers, Director

SUBJECT: LOBUND-ONR Report, 1 January 1953 - 30 June 1953

DATE: 31 July 1953

During the period covered by this report LOBUND's physiology and pathology laboratory faced the same questions and attempted to make contributions to the same problems as were outlined in some detail in this section of the previous report. In this task, as repeatedly stated, the description of animal life under gnotobiotic conditions occupies the central position. Additional responsibilities of this laboratory are service and collaborative functions. Thus, under these auspices, the following has been achieved during the past six months.

The quantitative anatomical and hematological data of germfree and conventional chickens prepared for publication in LOBUND Reports No. 3 have been evaluated from the viewpoint of (a) body-weight, organ weight relationship and (b) variability, in addition to the already performed other statistical analyses. Furthermore, considerable amount of time has been devoted to editorial and art work details of LOBUND's future publication.

New experimental data have also been accumulated for our germfree animal survey. As surplus animals were removed from the germfree colony or accidentally contaminated, previously germfree cages were broken up, efforts were made to evaluate these animals as fully as possible (gross and quantitative anatomy, hematology, histology and leukocytic balance studies). While these animals, as far as their backgrounds were concerned, did not necessarily fit into a properly laid out pattern, we hoped and to some extent succeeded in obtaining through them valuable data for our survey projects. Thus, during the past six months we have evaluated primarily: germfree rats; rats with previous germfree experience and in the process of adaptation to the conventional polyflora (i.e., germfree rats brought into our conventional colony); rats fully adapted to a mono- and di-flora.

LOBUND's commitment to the AEC has resulted in the study of a number of irradiated germfree and conventional rats. Besides these, a few additional experiments were run with irradiated rats, which are summarized in a special chapter of this report.

Finally, a status report of the amebiasis experiment (in collaboration with NIH) and a summary of the service functions of this laboratory are given.

A. Survey of Gnotobiotic Animals:

1. Morphological Description of Germfree and Conventional Chickens:

As reported, one of the criteria used by us in the characterization of gnotobiotic life is the comparison of organ weights among animals belonging to various experimental categories. In the first approach of this work such comparison was performed with animals grouped according to their age. While this method of grouping is generally used and accepted, thepossibility of an error is introduced, if (a) only few animals participate in one group, and (b) if within one age group the bodyweight is not particularly constant. Due to the fact that such conditions occasionally do exist in our experimental material, it appeared advisable to parallel our previous statistical analyses with the evaluation of groups which were formed on the bodyweight basis. A comparison between the germfree and conventional bantam chicken data summarized according to two bodyweight and two comparable age groups is given in Table I.

Table I. Organ Weight Analysis of White Wyandotte Bantam Chickens in Bodyweight and Age Groups

		Bodyweight Groups								Age Groups							
		125 - 250 gm				250 - 375 gm				30 - 37 days				50- 64 days			
		n	M	σ	p	n	M	σ	p	n	M	σ	p	n	M	σ	p
Spleen mg%	A	13	140	42	<0.01	7	150	25	<0.01	12	133	45	<0.01	13	164	44	0.04
	B	13	210	56		7	220	40		12	208	57		14	203	46	
Proventriculus Mg%	A	12	430	67	<0.01	7	340	51	1.00	11	503	118	0.10	13	350	58	0.04
	B	13	530	79		6	340	78		12	578	78		13	438	127	
Gizzard mg%	A	12	2000	393	<0.01	6	2140	304	0.64	11	2150	420	0.09	12	2200	660	0.09
	B	12	2620	520		7	2300	705		12	2800	1090		14	2820	1010	
Small Intestine mg%	A	13	2230	515	<0.01	6	1490	209	0.03	12	2550	630	0.01	13	1550	415	<0.01
	B	13	3600	1180		7	2220	612		12	3550	990		14	2990	1290	
Ceca, empty mg%	A	13	240	52	<0.01	7	180	33	<0.01	12	276	64	<0.01	13	200	51	<0.01
	B	12	350	65		7	280	32		12	360	62		13	315	76	
Cecal contents mg%	A	12	470	160	0.22	6	800	354	0.15	12	47[illegible]	185	0.13	13	699	367	0.24
	B	12	350	268		6	450	319		12	340	200		14	516	381	
Trident mg%	A.	13	37	9	<0.01	7	27	5	0.02	12	41	7	<0.01	13	28	5	<0.01
	B	13	66	29		7	68	38		12	62	17			70	37	
Bursa mg%	A	13	180	46	0.01	7	190	65	0.03	12	173	60	0.01	13	194	67	<0.01
	B	13	280	103		7	310	98		12	266	99		12	334	66	

TABLE I (Cont)

		Bodyweight Groups								Age Groups							
		125 - 250 gm				250 - 375 gm				30 - 37 days				50 - 64 days			
		n	M	o	p	n	M	o	p	n	M	o	p	n	M	o	p
Liver mg%	A	13	3160	536	0.05	7	2630	226	<0.01	12	3670	1150	0.37	13	2760	360	<0.01
	B	13	3760	829		7	3470	580		12	4070	884		14	3550	642	
Pancreas mg%	A	13	270	44	0.02	7	200	56	0.52	11	292	34	0.01	13	227	55	0.11
	B	13	340	85		7	220	49		11	364	72		14	277	94	
Lungs mg%	A	13	510	101	0.78	7	420	42	0.15	12	540	115	0.98	13	450	61	0.43
	B	13	500	72		7	470	63		9	541	86		14	471	66	
Heart mg%	A	13	550	71	1.00	7	380	54	0.68	12	603	114	0.53	13	454	85	0.89
	B	13	550	113		7	400	101		12	577	72		14	461	135	
Thyroid mg%	A	12	6.7	1.9	0.82	6	5.2	0.9	0.16	11	7.0	1.8	0.10	11	5.7	1.8	0.66
	B	11	6.5	2.0		6	4.2	1.1		11	5.6	1.7		12	5.3	2.1	
Thymus mg%	A	13	420	54	0.19	7	500	88	0.42	12	378	73	0.07	13	484	76	0.15
	B	13	360	143		7	440	146		12	308	98		14	407	162	
Adrenals mg%	A	13	14.3	4.2	0.09	6	8.7	1.6	0.53	12	17.2	5.3	0.08	12	10.3	2.2	0.82
	B	13	11.6	3.1		7	9.4	1.9		11	154	4.2		14	10.5	2.9	
Ovary mg%	A	7	32.0	88	0.74	6	31.0	4.2	0.60	6	27.1	5.8	0.09	10	34.3	5.8	0.88
	B	11	34.0	5.5		4	29.0	7.6		8	33.7	6.1		10	33.8	4.9	
Brain mg%	A	13	1070	161	0.18	7	710	77	0.89	12	1230	232	0.1.	13	750	142	0.08
	B	13	1170	178		7	700	60		12	1360	153		14	910	250	
Eyeball left mg%	A	13	460	75	0.06	7	340	24	0.23	12	532	125	0.12	13	361	52	0.05
	B	13	530	86		7	360	32		12	599	61		14	431	104	

A - germ-free, semi-synthetic sterilized diet, germ-free cage environment.
B - conventional contaminated, same diet, brooder room environment.
n - number of animals
M - arithmetic mean
o - standard deviation
p - statistic p (difference is assumed to be significant if p is less than 0.05).
mg% - mg per 100 gm bodyweight.

Table I shows in essence, that both germfree and conventional characteristics as revealed by the analysis performed on "age basis" is almost completely paralleled by the "bodyweight basis" grouping of the animals. Most external milieu organs (including the liver) of the germfree appear in both instances significantly lower in relative weight than the conventionals, while there is no such difference between the opposing animal categories in respect of the organs of the internal milieu. We regard the results of this analysis as a corroborative proof of our previous characterization of the germfree chicken.

2. Analysis of Variability of Morphological Data in Germfree and Conventional Chickens.

Routine autopsies performed in germfree and conventional animals over a period of years indicated that the former usually present a more uniform picture than their contaminated counterparts. This impression was obtained chiefly when growth, the relative size of various organs and some hematological data were recorded. By higher degree of uniformity in germfree it is meant that here the scattering of the numerical observations fell within a narrower range than in the conventionals.

Pointing towards a plausible and interesting difference between the various animal categories, it was decided to follow up the question in some detail. As the criterion of uniformity, the coefficients of variability (standard deviation expressed as the percentage of the mean) were calculated. The animal material used in this analysis originates from our bantam chicken survey, where a proper number of animals in adequate grouping seemed to be insured. Table II presents a compilation of the coefficients of variability assembled in three age groups and two bodyweight groups. Each value represents a total of 7 - 12 animals.

Table II. Coefficients of Variability of Growth, Hematological and Organ Weight Data in Bantam Chickens (White Wyandotte)

		Age Groups			Bodyweight Groups	
		30 - 34 days	50 - 70 days	150 - 170 days	125 - 250 gms	250 - 375 gms
		%				
Total Chicken	A	22.4	18.7	11.9		
	B	16.3	28.7	19.9		
RBC	A	10.7	17.9	11.1		
	B	11.5	15.8	20.2		
Hemoglobin	A	18.3	11.6	12.5		
	B	22.2	13.2	13.7		
WBC	A	47.7	31.1	22.7		
	B	44.5	52.2	32.6		
Heterophil BC	A	54.4	48.8	55.2		
	B	39.7	72.6	48.5		
Lymphocyte BC	A	50.6	40.0	22.1		
	B	49.2	53.4	38.9		

Table II (Cont)

		Age Groups			Bodyweight Groups	
		30 - 34 days	50 - 70 days	150 - 170 days	125 - 250 gms	250 - 375 gms
		%				
Clotting Time	A	89.6	63.3	25.5		
	B	85.8	58.2	24.5		
Spleen, wet	A	33.8	26.8	36.4	30.0	16.7
	B	27.4	22.7	34.6	26.7	18.2
Spleen, dry	A	17.0	9.3	5.1		
	B	15.7	7.3	7.4		
Muscle, dry	A	17.2	6.2	10.2		
	B	18.3	8.7	10.6		
Proventriculus, wet	A	23.5	16.6	25.9	15.6	15.0
	B	13.5	30.0	26.9	14.9	22.9
Proventriculus, dry	A	12.9	5.3	6.6		
	B	13.4	7.1	6.7		
Gizzard, wet	A	19.6	29.8	27.5	19.6	14.2
	B	38.9	35.7	16.7	19.8	30.7
Gizzard, dry	A	13.8	7.2	5.3		
	B	7.8	10.1	8.4		
Small Intestine Wet weight	A	24.8	26.8	14.5	23.1	14.0
	B	27.8	43.0	15.8	32.8	27.6
Small Intestine Weight dry	A	13.7	10.5	5.1		
	B	11.5	9.3	7.1		
Ileocecal tonsil Wet	A	17.1	17.9	5.0	24.3	18.5
	B	27.4	52.9	26.8	42.6	55.9
Cecum, empty, wet	A	23.2	25.5	25.2	21.7	18.3
	B	17.2	24.1	14.3	16.8	11.4
Cecum, empty, dry	A	19.5	7.4	5.7		
	B	14.5	11.5	12.4		
Cecal contents, wet	A	39.2	52.5	66.4	34.0	44.3
	B	58.8	73.8	40.5	76.6	69.3
Cecal contents dry	A					
	B					
Liver, wet	A	31.3	13.7	4.5	16.9	8.6
	B	21.7	18.1	22.4	22.0	16.7

Table II (Cont)

		Age Groups			Bodyweight Groups	
		30 - 34 days	50 - 70 days	150 - 170 days	125 - 250 gms	250 - 375 gms
		%				
Liver, dry	A	17.0	8.3	3.3		
	B	9.1	7.2	8.7		
Bursa, wet	A	34.7	34.5		25.6	34.2
	B	37.2	19.8		36.8	31.6
Bursa, dry	A	12.7	9.2			
	B	6.6	9.0			
Pancreas, wet	A	11.6	24.2	4.4	16.3	26.0
	B	19.8	33.9	17.9	25.0	22.2
Pancreas, dry	A	14.1	7.8	10.6		
	B	10.1	7.1	12.3		
Heart, wet	A	18.9	18.7	26.8	12.9	14.2
	B.	12.8	29.3	9.7	20.5	25.2
Heart, dry	A	11.8	5.6	7.8		
	B	7.1	7.7	8.7		
Lungs, wet	A	20.9	13.6	13.2	19.8	10.0
	B	15.9	14.0	23.1	14.4	13.4
Lungs, dry	A	18.6	14.5	5.4		
	B	14.5	14.2	7.5		
Thyroid, wet	A	25.7	31.6	16.9	28.4	17.3
	B	30.4	39.6	22.9	30.8	26.2
Thymus, wet	A	19.3	15.7	22.8	12.9	17.6
	B	31.8	39.8	27.4	39.7	33.2
Thymus, dry	A	17.2	7.1	5.7		
	B	15.3	4.0	13.0		
Adrenals, wet	A	30.8	21.4	19.6	29.4	18.4
	B	31.3	27.6	16.4	26.7	20.2
Kidneys, dry	A	14.8	10.3	7.4		
	B	15.2	8.3	9.5		
Ovary, wet	A	21.4	16.9		27.5	13.5
	B	18.1	14.5		16.2	25.2
Ovary, dry	A	26.0	11.1			
	B	29.6	14.4			

Table II (Cont)

		Age Groups			Bodyweight Groups	
		30 - 34 days	60 - 70 days	150 - 170 days	125 - 250 gms	250 - 375 gms
		%				
Brain, wet	A	18.8	18.9	12.11	15.0	10.8
	B	11.3	28.6	18.4	15.2	8.6
Brain, dry	A	16.7	5.9	6.6		
	B	14.0	5.9	5.2		
Eyeball, left	A	23.5	14.4	19.3	16.3	7.1
	B	10.2	24.1	12.3	16.2	8.9
Small Intestine length	A	10.9	11.5	9.1	10.6	7.3
	B	11.7	10.0	9.3	13.7	7.7

A and B - same as indicated in Table I
wet - values were calculated from wet organ weights expressed in mg per 100 gm bodyweight
dry - values were calculated from dry organ weights expressed in percentage.

The results show that in general the germfree (A) values are more stable (lower coefficients of variability) than the conventionals (B) in the following cases: (1) in the wet weights of organs which in normal life are in direct contact with the contaminating flora (i.e. most parts of the intestinal canal, including the liver and pancreas as well as the lungs); (2) lymphocytic organs which normally have flora contacts (the ileocecal tonsils including the circulating lymphocytes). Surprisingly, the thymus of germfree birds also shows more stable values. In contrast to these, there was no appreciable difference in variability between the internal milieu organs (including endocrines) of germfree and non-germfree birds.

These characteristics were clearly discernible (with few exceptions only) in both age and bodyweight type groups. They were manifest primarily in the older birds while the younger ones usually showed uncertain values.

In concluding this evaluation it can be stated that the analysis of variability produced such data which in our opinion are both plausible and support the evidence presented by the organ weight study itself. The lack of a bacterial impact in the external milieu and defense organs of the germfree apparently results in the lower degree of variability of this area in general when compared to the conventionals. On the other hand the protected internal milieu of both germfree and

conventionals fails to show any differences in this respect between the two. One of the most notable and hitherto unsuspected exceptions from this rule (which needs further proof) was the thymus of the germfree which with its weight, thymocyte concentration and "internal milieu protection" identical to the conventionals, still responded with a considerably lower degree of variability than the latter animal category.

Finally it appears necessary to emphasize that the data given in this report should not be generalized at this writing but refer only to the specific source of information. As preliminary data indicate, experiments performed with other species, diets, age and bodyweight groups, etc., may result in differences of the pattern described above.

3. Leukocyte Balance Studies.

In the initial phase of our endeavor to describe the animal organism in various forms of gnotobiosis, we have studied, among others, certain details and mode of response of the reticuloendothelial system. We have, for example, established in various organs of germfree and contaminated animals the concentration of leukocytic elements and their quantitative variation which follows environmental changes (such as the transition from germfree to mono-, di-, poly-contaminated state). In this approach, efforts were also made to correlate the type of leukocytic response to the nature (and combination) of the contaminant(s). Several phases of this work have already been reported to ONR.

However, the data of cell-distribution, while valuable from the viewpoint of general orientation, lack important details which appear indispensable to us for the clarification of the mentioned phenomenon. One detail is the release and utilization of reticuloendothelial elements as they are characteristic of or activated under these environmental conditions. Prerequisite to such balance studies is that methodologically they should indicate rather refined details of the cell response both on the organism and organ level. The other detail which needs further elucidation is to study the reticuloendothelial elements in the acquisition of their defense potential as well as in their performance of their specific functions.

During the past semester we have continued our leukocytic balance studies which were briefly described from the technical viewpoint in our previous report. As an illustration of the progress made, Figures 1 and 2 show the concentration of neutrophils and lymphocytes in various blood samples obtained from (a) germfree, (b) previously germfree, then contaminated by the normal polyflora, (c) normal conventional rats. By sacrificing three (b) type animals 1, 2 and 3 weeks following the gross contamination, our figures also show in some detail the transition from germfree to contaminated state.

There are many interesting details in this preliminary experiment for which Figures 1 and 2 should be directly consulted. Among these the following seem to be of special importance: (a) a rather spectacular

Figure 2. Concentration of lymphocytes in arterial and venous organ blood samples (cells per cu. m.m. ⌶ 2000 cells), each column of bars represents one rat.

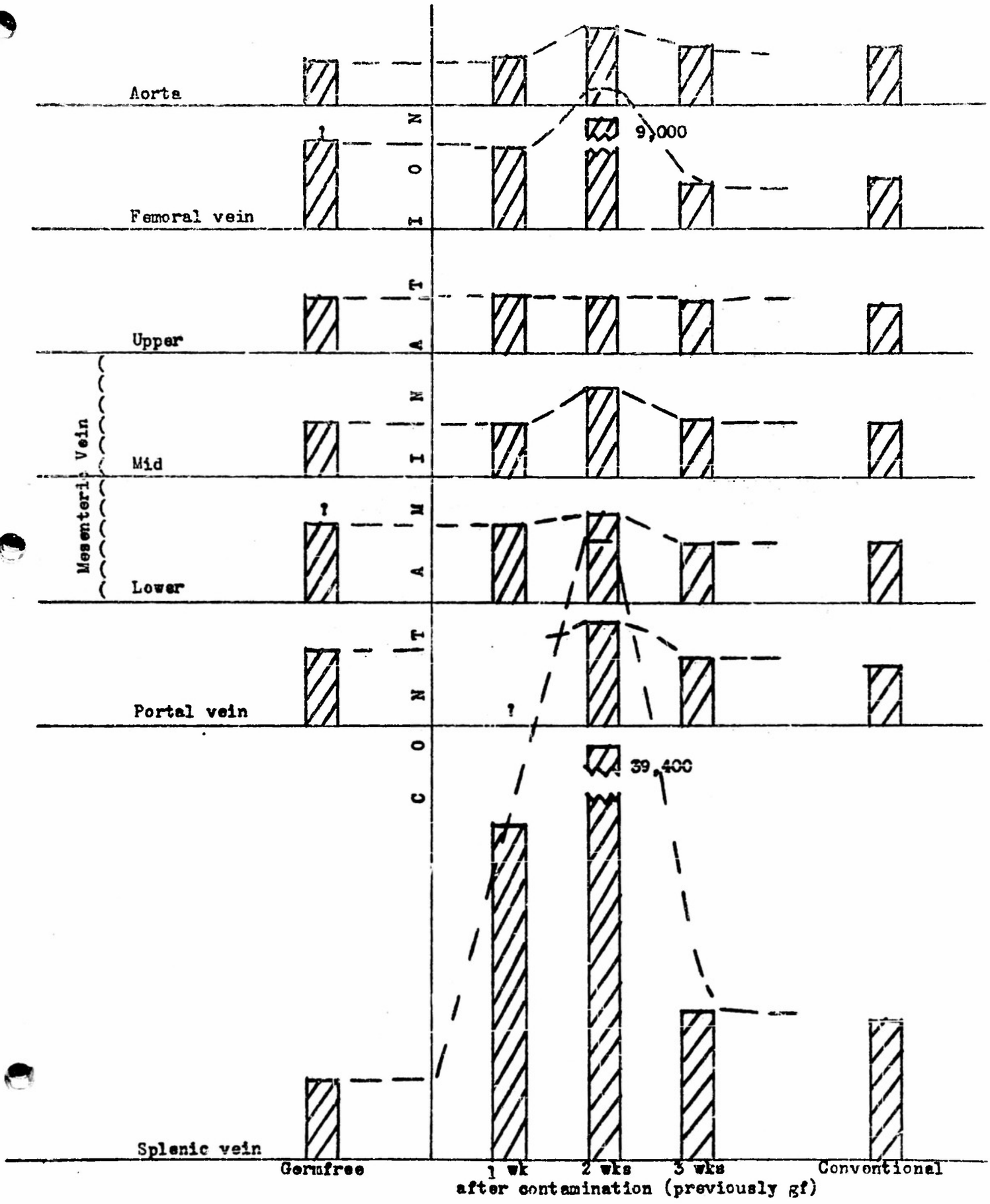


Figure 1. Concentration of neutrophilic granulocytes in arterial and venous organ blood samples (cells per cu. mm., I 2000 cells). Each column of bars represents one rat.

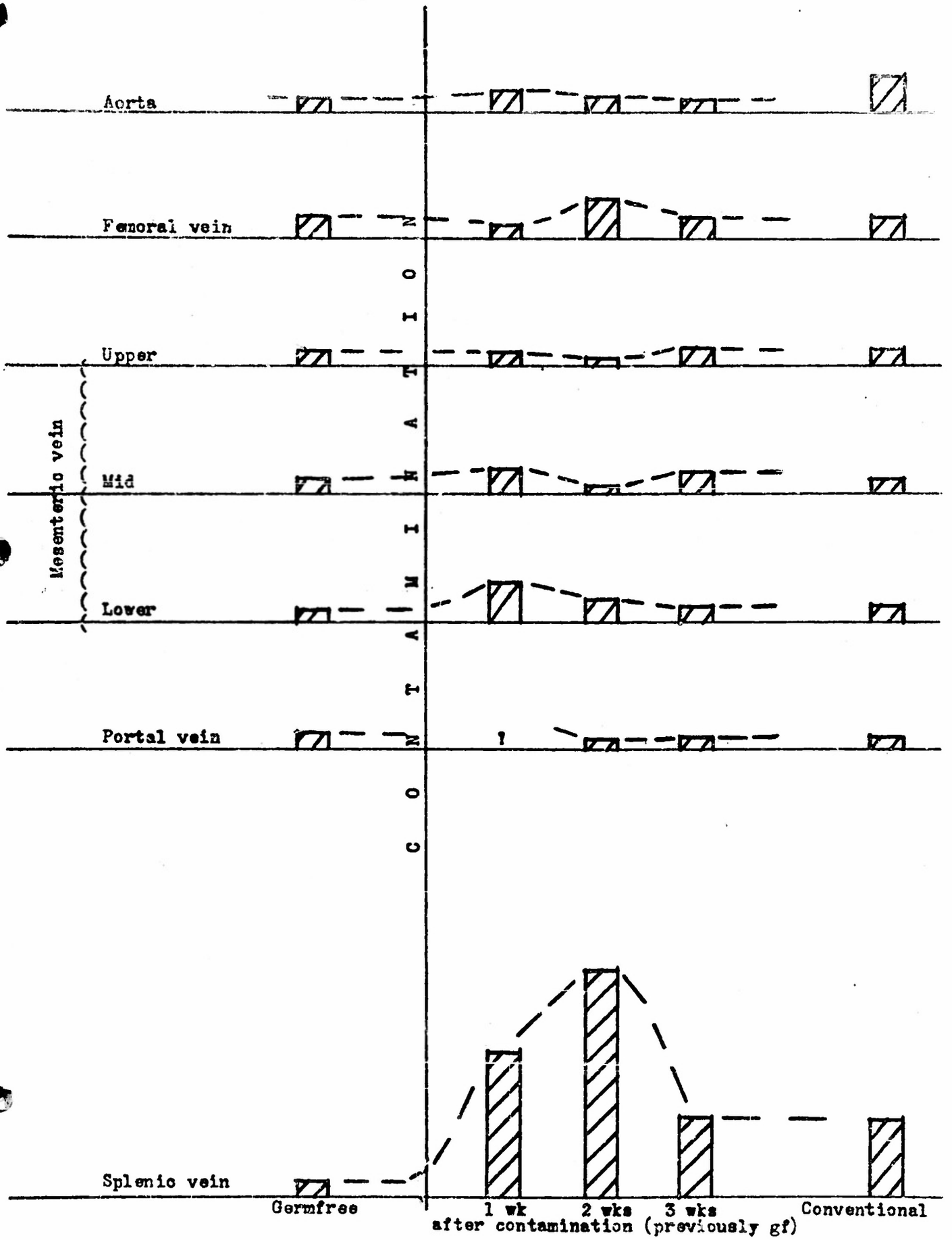
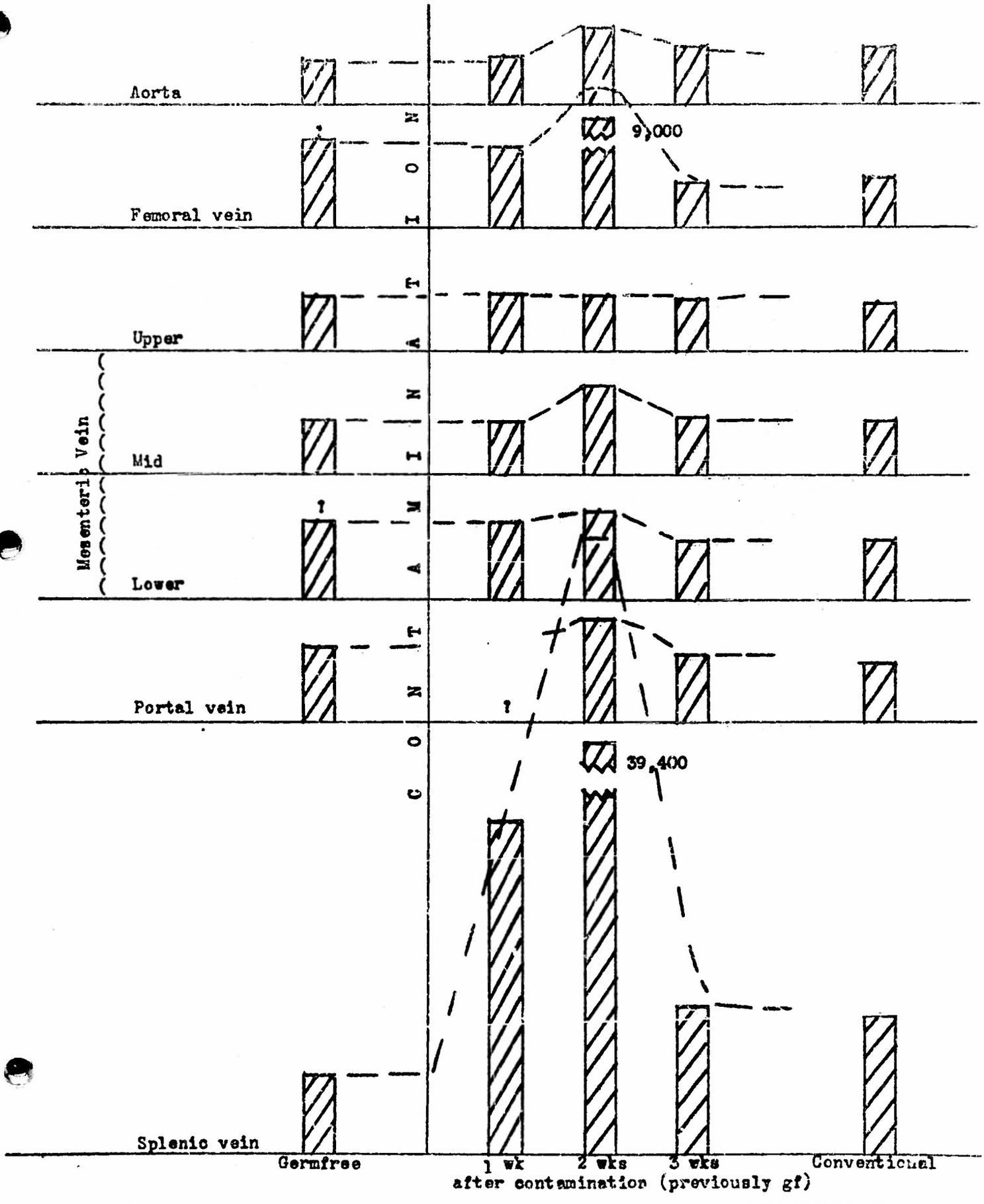


Figure 2. Concentration of lymphocytes in arterial and venous organ blood samples (cells per cu. m.m. ⌶ 2000 cells), each column of bars represents one rat.



increase of both granulocyte and lymphocyte output of the spleen during the weeks following contamination; (b) an increased utilization of granulocytes by the intestinal canal at the height of this response; (c) an increased output of lymphocytes in the lower segments of the small intestine (probably an expression of the already observed general lymphocytic hyperplasia following contamination); (d) within 3 weeks after contamination the mentioned responses subside and the previously germfree animal will stabilize with leukocytic levels which are comparable to the conventional rat.

B. Study of Comparative Effects of Total Body X-irradiation.

A rigorous analysis of our dosimetric factors was deemed necessary when a comparison of our dose-mortality curves for both germfree and conventional rats and those derived by other investigators showed that we never have 30 day survivals among our strain of rats when they have been exposed to more than 700 roentgens of total body x-irradiation while the other workers all have a number of survivors at this dose level. This observation was confirmed both by direct communication with various radiation biologists as well as by a search of the literature.

A survey of more than 150 published papers dealing with the acute radiation syndrome and other radiation effects showed that few investigators adequately stated their dosimetry (in terms, for example, of added filtration, type of x-ray unit, and dose-rate) and almost none of them stated the effective wave-length used or its equivalent in terms of half-value layer. Lack of such statements precludes any valid comparison of results between various laboratories. This is a view held not only by ourselves but also by the Naval Radiological Defense Laboratory and by Dr. F. Ellinger (1), Chief Radiation Biologist at the Naval Medical Research Institute and his colleagues. Of the few papers which do adequately give the dosimetry used, it was found that these investigators have used x-ray beams with half-value layers 1/4 to 3/4 the thickness of our own which is slightly greater than 2.9 mm. Cu. In Addition, our animals are exposed to irradiation while confined in aluminum restraints while no other worker in the field appears to use anything other than restraints made of wood or plastic. This fact alone shows that our animals have received, in addition to the harder components emitted by the x-ray tube, fluorescent x-rays from the aluminum. These fluorescent x-rays according to Trump (2) increase the ion-density in tissues and structures close to the surface of the skin; accordingly, our animals must be affected over a wider cross-sectional area of their bodies by the x-rays and therefore our dose-response curves must be expected to be significantly different from those of other investigators inasmuch as the biological effect of x-irradiation is dependent on the ion-density produced.

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1. Ellinger, F.: Pharmacological Studies on irradiated animals: Part I; Scope and Methodology. Research Report, Project NM 006 012.05.04, 10;331-334, 28 May 1952.

2. Trump, J. G.: Physical basis for the high skin tolerance of supervoltage roentgen rays. Radiology, 50(5):649-655, 1948.

In addition to the dosimetric analysis made during the past 6 months we have continued to collect data to complete our dose-mortality curves comparing the effects of total body x-irradiation on germfree and LOBUND strain conventional rats. This was agreed to be necessary at the AEC-LOBUND-ONR Advisory Committee meeting held at the University of Notre Dame in January of 1953 inasmuch as it was felt that an insufficient number of rats had been exposed to doses in the 400 roentgen to 600 roentgen dose range. In addition it was felt that it would be of considerable interest if we could collect additional data concerning the effect of supralethal total body x-irradiation on mono- and dicontaminated, formerly germfree rats. Details of this and other projects involving the use of germfree animals in the study of the acute radiation syndrome will be separately submitted at a later date.

C. Investigations on the Influence of the Intestinal Flora on the Infectivity and Pathogenicity of E. histolytica, (with Dr. W. Wright, Dr. C. Rees and B. Phillips (in residence at LOBUND) of the Microbiological Institute at NIH). Report by B. Phillips.

The collaborative project with the Laboratory of Tropical Diseases of the National Institutes of Health and the LOBUND Institute of the University of Notre Dame which has as its objective a study of "The role of the intestinal flora in the production of acute amoebiasis" was continued during the first half of the year 1953. Some additional data were obtained and considerable progress was made in the elimination of the innumerable factors that have hampered efforts to effectively pursue the problem from the time of its initiation.

Previous reports listed and discussed briefly some of the many complications attending the problem as well as the methods which were employed in attempts to eliminate such complications that the studies might continue at an accelerated rate. However, some of these complications have proved to be major research problems in themselves and have required considerable attention which has at times detracted necessarily from the amoebiasis investigations per se.

Although the data obtained up to the present time are too limited to furnish the basis for any definite unequivocal conclusions, it suggests that E. histolytica is dependent in some way upon the activity of bacterial associates for its disease-producing properties. The specific mechanism, however, of such activity remains obscure. The data do not as yet show that such bacterial activity contributes directly to the pathogenicity of the ameba rather than merely to its infectivity. Since the amebas fail to survive for prolonged periods in the germfree intestine, the possibility arises that bacterial associates may contribute only by sustaining growth of the ameba until such time as it is capable of penetrating the walls of the intestine. An answer to the question relative to bacterial activity contributing to pathogenicity versus infectivity must await, at least, the anticipated studies with animals harboring monobacterial contaminants.

Of considerable importance to the project is the fact that the nutrition of germfree guinea pigs is no longer an obstacle to these investigations. Numerous diets have been tested and a diet has been developed that permits survival and growth of animals of good quality for periods

of time considerably in excess of the requirement of the amoebiasis experiments. Furthermore, the use of the same sterile diet in conventional control animals permits high grade amoebic infections with accompanying lesions.

Of similar importance to the investigations is the fact that previous problems of contamination in germfree guinea pigs seems to have been dealt with effectively in that no contamination has been experienced in this series during the past four months.

The problem of anesthesiology in germfree guinea pigs, which, incidentally, is a problem of considerable magnitude in itself, appears to be responding favorably to efforts to alleviate it. Past attempts at inoculative surgery of the germfree pigs have been characterized by a heavy loss of animals that have been reared successfully to the age whereby they were suitable for inoculation; this loss has invariably been due to undesirable effects of the anesthetic (Nembutal). Although many of the animals regain consciousness within a few hours of the surgery and experience no complications that may be attributable to the surgery per se, they fail to return to vital capacity and the barbiturate appears to inhibit their normal metabolic activity for a period sufficiently long to prohibit their full recovery and survival. Animals that die show symptoms of inability to eliminate waste materials from the intestines and kidneys; this has not been relieved by administration of adrenalin and other stimulants or by attempts at irrigation of the intestine. More recent tests with other steam sterilized anesthetics in 3 days-old conventional guinea pigs show considerable promise of obviating this major complication. Pentothal sodium is most promising. This barbiturate seems to produce adequate anesthesia and its effects are of considerably shorter duration than Nembutal. Furthermore, in guinea pigs, the margin between adequate dosages and toxic dosages seems to be considerably greater than with Nembutal. The use of pentothal sodium followed by the administration of Picrotoxin as an analeptic has produced some remarkable results as compared to the results previously obtained with Nembutal.

In the immediate future it is planned to expand the data in the germfree series to a point where it is available on a minimum of 15 to 20 animals that have survived for at least the 21-day post-inoculation period. If all data thus obtained are negative, it is planned to inoculate monobacterial contaminated pigs in the hope of answering the question as to whether specific bacteria (for example pathogenic organisms) are essential to the disease producing qualities of the ameba or whether non-pathogenic organisms such as organism t and the S-F streptobacillus, which should sustain vigorous amebic growth in the intestine, are all that are required to render this so-called pathogenic ameba a pathogen.

D. Service Function:

The principle service function of this laboratory is that of performing autopsies and clinico-pathological procedures for the germfree animal production and stock animal colony teams. In addition to this service in the last half year we have attempted to sterilize

by x-irradiation a group of samples of ascorbic acid as an aid to Dr. E. Hawk of NIH who is working as a collaborator in the Biochemistry-Nutrition Laboratory* and we have proceeded to establish a functioning radiographic service as a diagnostic aid (in the question of cecal volvuli in germfree rats, for example), as an aid in the dental caries project, and to facilitate the selection of timed pregnancy mammals for delivery by Caesarian section into the germfree system. In this last matter, routine radiography of pregnant guinea pigs supposedly at term has resulted in the delivery of larger, healthier guinea pigs into the germfree system and the saving of considerable time and cost to the personnel concerned with the amoebiasis project.

Relative to autopsies performed for the germfree production team we continue to find frequent intestinal volvuli. In addition, we find an occasional rat dead of the "lung syndrome" which has been diagnosed as hemorrhagic bronchopneumonia. As stated in the last report submitted by this laboratory, we have continued to search for a possible physical etiological agent for this condition, and recent findings indicate that at least in some of the cases seen, the pulmonary lesion may result from a traumatic injury to the central nervous system. We were given a clue to this possibility when Dr. E. Hawk, in the routine sacrifice of some of his rats by "cervical fracture" noted an extremely severe pulmonary edema which was not seen when the animals were disposed of through an overdose of chloroform, ether, or pentobarbital anesthesia. Our belief that cerebral trauma might be the cause of some of the changes seen in our rats was fortified by the fact that MacKay has produced pulmonary edema experimentally by cerebral injuries. We are devising pharmaco-physiological experiments to test this possibility methodically.

We have processed (for morphological anatomical survey a) or performed autopsies on a total of 485 animals, exclusive of those used in the amoebiasis study, including 389 rats, 10 mice, 52 chickens, 16 turkeys, and 18 miscellaneous larger mammals.

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* A detailed report of this activity is presented in the Bacteriology Section (Section IV).

VI. VIROLOGY

FROM: J. F. Rebsak, Virologist
(with the assistance of M. Sacksteder)

TO: J. A. Reyniers, Director

SUBJECT: LOBUND - ONR Report, 1 January 1953,- 30 June 1953

DATE: 31 July 1953

A. Introduction:

Is the "germfree" animal free of viruses? Will the (otherwise) germfree animal respond to virus infection in a different way than the conventional "contaminated" animal does? Might the germfree animal prove responsive or susceptible to agents to which the conventional animal is not; and/or vice versa?

How well can we answer these questions for the present?

To date we have seen no clear indication of the "natural" occurrence of viral pathogens in the germfree animal. The available evidence allows little more than debatable interpretation as regards, for instance, (a) egg-transmission of virus into the germfree biotope (as, possibly, germfree chicken "jitters"), or (b) occurrence of agents inducing host-cell proliferation (again, "jitters"), or (c) occurrence of agents causing hemorrhagic response ("lung condition" in germfree rats). Presence of virus in these situations is still only a theoretical possibility.

Symbiotic viruses or virus-like agents, if such exist, so far escape detection, or may simply be lost in the present-day hazy semantics of this developing biological subdivision. Better resolution is needed. What, for example, is the mechanism of viral genesis of tumor? Of bacterial mutation by phage? Do "latent" viruses change into "active" host genes? Can host gene mutate to free lance virus? And if actual transformation does not occur, how then does the exogenous, invading virus particle contrive to influence, or even dominate, the hereditary apparatus of the host?

The coming era may well find the virus and the gene classified together, no more different than the "farmer" and the "dweller in the metropolis." Under necessity to return to the present, however, and to problems at hand, we once again resort to our "working hypothesis" (seemingly still valid): the postulate that the germfree animal at least appears to be virus-free (cf. LOBUND-ONR Report for 31 July 1952).

Production of the germfree animal is characterized by curtailment of antigenic stimuli. The resultant living milieu might be expected to be more vulnerable, than antigen-educated animals, to any manner of aggressive agent or non-living irritant. This has been found to be true, as regards replicating agents, previously in the discovery of the

occurrence of bacteremia in germfree chicks with such organisms as Bacillus subtilis, Alcaligenes fecalis, Staphylococcus albus, and Sarcina sp.,* and recently as regards the shortened incubation period, in germfree chicks, with the viruses of Newcastle disease and Rous sarcoma (cf. LOBUND ONR Reports for 31 January 1952 and 31 July 1952, respectively).

Perhaps no better answers than these may be given to the first two questions of our opening paragraph. This brings us to the last: Might the germfree animal respond to agents to which the conventional animal does not? or vice versa?

Here we might extract some data from experiments currently underway in our laboratory. We are studying the effect on germfree chickens of ultrafiltrates (1) of sera from patients with infectious hepatitis, (2) of sera from patients with Hodgkins disease, and (3) of methylcholanthrene-induced tumors in chickens. Conventionally-reared laboratory hosts have not proved responsive in analogous studies.

On the other side of the question, we are proceeding with observations on the effect of methylcholanthrene on the germfree chicken. Use of this carcinogen in conventional birds assures tumorigenesis. It may be recalled** that our real interest in the use of MChA in germfree animals is based on the possible liberation of a virus by such agents. In the near future, sera from both germfree and conventional chickens bearing MChA-induced tumors will be tested for possible antibody levels against such antigens as the virus of Rous sarcoma, etc.

As a further possible contribution toward the measure of comparative susceptibility of germfree and of conventionally-reared animals, we shall mention briefly, in the following pages, our tests on the action in germfree chickens of the filtrable agent of lymphomatosis, indeed a common (though incompletely understood) disease of barnyard poultry.

It is emphasized that the following data are drawn from unfinished experiments. Interpretations or deductions made at this time must be tentative. A fair amount of descriptive text relative to this work was incorporated in our last ONR Report (13 February 1953). Concentration in the present instance has been on the tabulation of our results up to now. Effort is made to present this tabulated material without excessive duplication of the textual material presented formerly.

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*N.B. - Reynolds, J. A., "Some observations on rearing laboratory Vertebrates germfree," Proc. N. Y. State Assoc. Publ. Hlth. Labs. 28:60 (1949).

**N.B.- Cf. LOBUND-ONR Reports for 25 July 1951 and 13 February 1953.

B. Human Infectious Hepatitis:

As previously reported, the object of this work may be given as: (1) to determine what activity, if any, the virus of human infectious hepatitis might show in germfree chickens at different ages, and (2) to determine whether incidence might be abetted by sub-lethal X-ray preparation of the host.

To be of use under germfree conditions, the infected human sera* must be subjected to ultrafiltration. This is done with ultrafine pore size fritted glass filters (1 - 2μ, labeled "UF"). Filtrates so prepared prove free of bacteria and molds when tested in a variety of broth and solid media. Unfortunately, no means other than the use of human volunteers could assure the presence of the hepatitis virus in such ultrafiltrates; so that in this respect we are obliged to work "blind".

We have already inoculated hepatitis ultrafiltrates, by various routes, into a number of germfree and conventional chickens at various age levels. Indication is given in Table I of the rate of incidence of a peculiar set of symptoms (syndrome "X") which we have observed to occur in a series of so inoculated (otherwise) germfree White Leghorn chickens. Syndrome "X" has been described previously. In brief resume, it may be said that generalized muscular debilitation and gastro-intestinal disturbance appear to be the more definitive effects. Regurgitation of crop contents is common. Afflicted birds become prostrate, then moribund. No constant pathology has been uncovered under gross or microscopic examination.

No effect from similar inocula has been noted thus far in conventional "contaminated chickens (Table II), though the number of valid observations here is small, and, as yet, not extended beyond 33 days post-inoculation.

Only negative results were also obtained with an even smaller group of germfree New Hampshire Red chickens (Table III), though the effect of early contamination of this group is difficult to assess.

Attempt has also been made to determine the effect, in relation to incidence of "infection" (syndrome "X"), of sub-lethal exposure of the inoculated or to-be-inoculated host to X-rays. Results with otherwise germfree chickens are given in Table IV, with conventional chickens in Table II. The limited data do not permit any clear statement as to the influence of irradiation.

In Table V we present evidence for a filtrable etiological agent for syndrome "X".

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*E.B.- Infectious hepatitis sera obtained through the courtesy of Drs. W. Henle and J. Stokes of the University of Pennsylvania.

Table I. - Effect of inoculation of ultrafiltrates of human infectious hepatitis sera on germfree White Leghorn chickens.

Chicken No.	Age (days)	Inoculation data: Route	Inoculation data: ml**	Incidence Syndrome "X"	Incubation period	Period of observation
3	76	IV	1.0	Positive	20 days	22 days
24	56	IV	0.5	Positive	42	45
435	28	Oral	0.35	Positive	9	12
436	28	IM	0.25	Positive	9	12
440	28	IM	0.25	Positive	12	15
16	56	IV	0.5	Doubtful	(51)	52
335*	31	IV	0.5	Negative	—	32
340*	31	IV	0.5	Negative	—	32
371	9	IV	0.5	Negative	—	60
404	8	IV	0.5	Negative	—	59
332*	31	Oral	0.25	Negative	—	32
333*	31	Oral	0.25	Negative	—	32
377	9	Oral	0.4	Negative	—	60
380	9	Oral	0.4	Negative	—	60
405	8	Oral	0.6	Negative	—	59
331*	31	IM	0.5	Negative	—	32
338*	31	IM	0.5	Negative	—	32
378	9	IM	0.5	Negative	—	3
378	9	IM	0.5	Negative	—	60
401	8	IM	0.5	Negative	—	59
334*	31	IP	0.5	Negative	—	32
337*	31	IP	0.5	Negative	—	32
379	9	IP	0.5	Negative	—	60
402	8	IP	0.5	Negative	—	59
339*	31	Contact	0	Negative	—	32

*N.B. - Contaminated with Escherichia coli between the 15th and the 16th day after inoculation with the hepatitis filtrate.

**N.B. - Of the filtrate of undiluted infected human serum.

Table II - Effect of X-ray irradiation and/or inoculation of filtrates of (undiluted) human infectious hepatitis sera (or plasma) on conventional White Leghorn chickens.

Chicken No.	Age (days)	Treatment					Incidence syndrome "X"	Period of observation (days)
		1	2					
		Radiation dosage	Inoculation data			Interval between 1 & 2		
			Route	Filtrate	ml			
929	52	438r	IV	Coarse	0.2	Same day	Negative	1**
935	52	438r	IV	Coarse	0.2	Same day	Negative	1**
948	35	400r	IV	Ultra	0.6	Same day	Negative	7**
949	35	400r	IV	Ultra	0.25	Same day	Negative	7**
992	42	400r	IV	Ultra*	0.5	2 days	Negative	35
999	42	400r	IV	Ultra*	0.5	2 days	Negative	35
921	52	—	IV	Coarse	0.2	—	Negative	1**
927	52	—	IV	Coarse	0.2	—	Negative	1**
945	35	—	IV	Ultra	0.6	—	Negative	7**
950	35	—	IV	Ultra	0.25	—	Negative	7**
103	42	—	IV	Ultra*	0.5	—	Negative	33
993	42	—	IV	Ultra*	0.5	—	Negative	33
930	52	438r	Contact	—	—	Same day	Negative	4**
933	52	438r	Contact	—	—	Same day	Negative	1**
938	35	400r	"	—	—	Same day	Negative	8**
948	35	400r	"	—	—	Same day	Negative	7**
101	42	400r	"	—	—	2 days	Negative	35
102	42	400r	"	—	—	2 days	Doubtful (28-31 days***)	35
924	52	—	"	—	—	—	Negative	1**
925	52	—	"	—	—	—	Negative	1**
943	35	—	"	—	—	—	Negative	7**
951	35	—	"	—	—	—	Negative	7**
104	42	—	"	—	—	—	Negative	33
996	42	—	"	—	—	—	Negative	33

*N.B. - Ultrafiltrates of human plasma, as distinguished from human serum in the other instances.

**N.B. - Early loss of birds due to air supply failure in isolation units.

***N.B. - "Incubation period", counted from initial contact exposure to inoculated birds.

Table III. - Effect of inoculation of an ultrafiltrate of pooled (undiluted) human infectious hepatitis sera on 30-day germfree* New Hampshire Red chickens.

Chicken No.	Inoculation data		Incidence syndrome "X"	Period of observation
	Route	ml		
451	IM	0.4	Negative	22 days
452	IM	0.4	Negative	22
453	IV	0.3	Negative	22
454	IV	0.3	Negative	22
455	Contact	0	Negative	22

*N.B.- Contaminated with a mold (Penicillium sp.) in 6 to 8 days following inoculation with the hepatitis filtrate.

Table IV.- Effect of X-ray irradiation and inoculation of ultrafiltrates of human infectious hepatitis sera on germfree White Leghorn chickens.

Chicken No.	Age (days)	Treatment*			Incidence syndrome "X"	Incubation period	Period of observation
		1	2	Interval**			
1	76	438r	1.0 ml, IV	Same day	Positive	12 days	
11	56	400r	0.3 ml, IV	2 days	Positive	38	
25	56	400r	0.3 ml, IV	2 days	Doubtful	(51)	52 days
2	76	1.0 ml, IV	438r	Same day	Positive	18	
457	28	0.25ml, IV	300r	54 days	Negative	—	89
456	28	0.25ml, IV	300r	54 days	Negative	—	89
459	28	0.25ml, IP	300r	54 days	Negative	—	89
13	56	400r	Contact	2 days	Negative	—	38
22	56	400r	Contact	2 days	Doubtful	(60)	63
28	56	Contact	—	—	Negative	—	38
30	56	Contact	—	—	Doubtful	(49)	50

*N.B.- Treatment consisted of: (1) X-ray irradiation and inoculation with the undiluted infected human serum, or (2) vice versa, or (3) X-ray irradiation alone, or (4) simply contact exposure to the inoculated birds.

**N.B.- Interval, in days, between manipulations "1" and "2".

Table V. History of germfree chicken passage of filtrable Agent "X" from human infectious hepatitis sera (Akiba strain).

Passage	Chicken strain (stock)	Age (days)	Incidence syndrome "X"	Incubation period	Period of observation
1st*	White Leghorn(Jones Bros.)	76	3/3	12-20 days	22 days
2nd	White Leghorn(Jones Bros.)	88	1/1	10-12 days	30 days
2nd	White Leghorn(Highview)	58	2/2(?)	(10-16 days)	18 days
3rd*	White Leghorn(Highview)	85	1/2	2-8 days	18 days
4th*	New Hampshire Red(Liechty)	32	0/3	---	21 days**

*N.B.- Ultrafiltrates inoculated.
**N.B.- Contaminated between 0 and 8 days after inoculation.

Table VI is a record of a number of sheep RBC agglutination (heterophile antibody) tests which we have conducted with preinfected, acute phase, and convalescent human sera and with normal, contact, 1st, 2nd, 3rd, and 4th passage chicken sera. If the 1:16 dilution of serum is taken as a non-specific base level, it may be seen that three of the inoculated birds show titres which compare favorably with the heterophile antibody levels in the acute phase and convalescent human sera. However, if sheep RBC agglutination is a test for "antibody", the high titres in the acute stage human sera (Table VI) are a little surprising.

Cephalin-cholesterol flocculation tests with these sera gave rather erratic and confusing results, and these will not be reproduced here.

It may perhaps be tentatively concluded that though there is indication in our results to date of some susceptibility of the germfree chicken to the agent of human infectious hepatitis, it seems rather unlikely that the germfree chicken will prove to be a highly satisfactory laboratory host for this agent.

However, much work might still be done. Our work should be repeated, with institution of further control procedures, such as the inoculation of heat-inactivated hepatitis sera, etc.

Is there indication here of different comparative susceptibility between the germfree and the conventional chicken? On an immunological basis, it is not difficult to accept the possibility of minor differences in germfree and conventional host susceptibility to a given agent; as, for example, relative incubation periods, relative tendency to viremia or localization of the infection, etc. But as regards the possibility of complete susceptibility of one and complete insusceptibility of the other, to a given agent, we would doubtless like to see evidence of a little more robust nature.

Table VI.- Agglutination of Sheep RBC by normal and hepatitis infected human and chicken sera. (Test for heterophile antibody).

Serum Sample	Description	Syndrome "Y"	Days post-inoc.	Dilution of Sera											
				1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192
Human A	Preinfection					+	±	±	-	-	-	-	-	-	-	
Human B	Preinfection					+	±	±	-	-	-	-	-	-	-	
Human C	Acute Phase					±	+	+	+	+	±	±	-	-	-	
Human D	Acute, C Filtrate					+	+	+	±	±	-	-	-	-	-	
Human E	Acute, C Filtrate					+	+	+	+	±	-	-	-	-	-	
Human E	Acute, UF Filtrate					-	-	-	-	-	-	-	-	-	-	
Human F	Acute, UF Filtrate					+	+	±	±	-	-	-	-	-	-	
Human G	Convalescent					+	+	+	±	-	-	-	-	-	-	
Human H	Convalescent					+	+	+	±	-	-	-	-	-	-	
Human I	Convalescent				+	+	+	+	+	+	±	-	-	-		
Conv. Chicken N1	Normal						-	-	-	-	-	-	-	-	-	-
Conv. Chicken N2	Normal						-	-	-	-	-	-	-	-	-	
Conv. Chicken N3	Normal						-	-	-	-	-	-	-	-	-	
Conv. Chicken N4	Normal			-	-	-	-	-	-	-	-	-	-			
Conv. Chicken N5	Normal					±	-	-	-	-	-	-	-	-	-	
GF Chicken 455	Uninoc., Contact	No	(22)				-	-	-	-	-	-	-	-	-	
GF Chicken 478	Uninoc., Contact	No	(21)			±	-	-	-	-	-	-	-	-	-	
GF Chicken 22	Uninoc., Contact	?	(63)				-	-	-	-	-	-	-	-	-	
GF Chicken 11	1st Passage, IV	Yes	38				-	-	-	-	-	-	-	-	-	
GF Chicken 24	1st Passage, IV	Yes	35				-	-	-	-	-	-	-	-	-	
GF Chicken 25	1st Passage, IV	?	52			+	+	+	-	-	-	-	-	-	-	
GF Chicken 435	1st Passage, Oral	Yes	11			+	+	+	+	+	+	±	-	-	-	
GF Chicken 440	1st Passage, IM	Yes	12				-	-	-	-	-	-	-	-	-	
GF Pool 451, 452	1st Passage, IM	No	22	+	+	±	-	-	-	-	-	-	-			
GF Pool 453, 454	1st Passage, IV	No	22	+	+	±	-	-	-	-	-	-	-			
GF Pool 5 birds	1st P., Oral, IV, IP, IM	No	60				-	-	-	-	-	-	-	-	-	
GF Chicken 4	2nd Passage, IV	Yes	30				±	-	-	-	-	-	-	-	-	-
GF Chicken 26	2nd Passage, IV	?	18			+	+	+	+	-	-	-	-	-	-	
GF Chicken 18	3rd Passage, IV	Yes	8				-	-	-	-	-	-	-	-	-	
GF Chicken 470	4th Passage, IM	No	21		+	±	-	-	-	-	-	-	-			
GF Pool 477, 479	4th Passage, IV	No	21		+	+	±	-	-	-	-	-	-	-		

Conv.: Conventional
GF: Germfree
Uninoc: Uninoculated
C: Coarse
UF: Ultrafine
IV: Intravenously
IP: Intraperitoneally
IM: Intramuscularly

C. Methylcholanthrene and Filtrates of MCHA-Induced Tumors:

Our aim here is to make an attempt at filtrate passage of methylcholanthrene-induced tumors in germfree chickens, on the change that this carcinogen may be inducing or liberating an active, transmissible virus-like principle. The experiments are of two types: (1) inoculation of methylcholanthren under germfree conditions, and (2) inoculation of cell-free material from the MChA-induced tumor growth. Inoculation and other technical procedures have been detailed in several preceding reports, including the last.

For the present, we have summarized our recent experiments and present these in tabular form. Table VIII is a record of results of experiments started, and in some instances maintained for considerable periods, under germfree conditions. Data are included from seven MChA inoculation experiments and three experiments with ultrafiltrates of two different pools of MChA-induced tumors (3 tumors per pool). Table VII is included in order to show the comparative effects of these same inocula in conventional "contaminated" chickens.

Table VIII shows that we have observed, so far, at least six instances of incipient tumorigenesis under bona fide germfree conditions. In its response to this carcinogen, the germfree chicken would seem physiologically not unlike its "contaminated" relative. If MChA function to release a viral entity, then this entity, or at least its pro-entity, must be present in the germfree as well as in the contaminated chicken. It may be seen (Table VIII) that many more of the birds, inoculated under germfree conditions, went on to show tumor growth after contamination had occurred. It seems an even chance that in these instances genesis of tumor, though not grossly detectable, began while the chicken was still in the germfree state.

From the results of our filtrate inoculation experiments (Table VII and VIII), it is apparent that, at this point, the evidence is against the possible generation of a virus-like agent by methylcholanthrene. At least, "it" would not appear to be transmissible.

This work is to continue.

D. Hodgkins Disease:

We recently undertook the study of the effect on our germfree chickens of sera obtained* from patients with this disease of the lymphatics. No suitable conventional laboratory "host" for this disease is known. It must be remembered again that under germfree conditions only ultrafiltrates of the pooled human sera may be used.

We have two germfree experiments to report, the first completed except for histopathology, the second currently under observation.

Experiment No. 61Ll-1: Methods and results (so far available) are summarized in Table IX. In addition to the effects on the thymus nodules recorded in the table, changes were noted, in one instance or another, in the spleen, the pancreas and the bursa. Sections are being prepared for microscopic examination. At present, further exact data on normal White Leghorn chickens is being collected to enable proper quantitative comparison of thymus-to-bodyweight ratios between these "infected" and normal chickens.

*N.B. - Courtesy of Dr. A. Rottino, St. Vincent's Hospital, New York City.

Table VII.- Tumorigenesis in conventional White Leghorn chickens with methylcholanthrene and with filtrates of pooled MChA-induced tumors.

Exp. No.	Chicken Stock	Age (days)	Inoculum	Tumorigenesis		
				Rate	Time	Percent
1*	Dembro	11	MChA	3/5	2-1/2 - 5 months	60
			None	0/2	5 months	0
2*	Highview	8	MChA	1/6	3-1/2 months	17
3*	Highview	8	MChA inoc. and painting	1/9	1 month	11
F1	Dembro	13	C Filtrate I	0/2	8 months	0
			UF Filtrate I	0/2	8 months	0
F2*	Highview	8	C Filtrate II	0/4	1 month	0
			UF Filtrate II	0/6	1 month	0

*N.B. - Current experiments, 13 July 1953.

Table VIII. Tumorigenesis in germfree White Leghorn chickens with methylcholanthrene and with filtrates of pooled MChA-induced tumors.

Exp. No.	Chicken stock	Age (days)	Inoculum	Tumorigenesis			Contam. (days post-inoc.)	Genesis under GF conditions
				Rate	Time	Per cent		
1	Highview	6	MChA	2/5	3 - 4 months	40	6	0
			Benzene	0/3	4 months	0	6	---
2	Highview	9	MChA	2/3	2 months	67	67	2
3	Dembro	5	MChA	2/5	2 - 3 months	40	71	1
4	Dembro	8	MChA	4/7	5 - 6 months	57	14-20	0
5*	Highview	8	MChA	4/8	2½ - 3½ mos.	50	0	0
6*	Highview	9	MChA	3/5	2 months	60	68-74	3
7*	Highview	8	MChA inoc. & painting	(1)/7	1 month	(14)	GF	(1)
F1	Dembro	13	UF Filtrate I	0/3	7 months	0	8-14	0
			None	0/2	7 months	0	8-14	0
F2*	Highview	8	UF Filtrate II	0/4	2 months	0	GF	0
			Water	0/2	2 months	0	GF	---
F3*	Highview	8	UF Filtrate II	0/6	1 month	0	GF	0
			Water	0/1	1 month	0	GF	---

*N.B.- Current experiments, 13 July 1953.

Experiment No. 61L1-2: Ultrafiltrates prepared of serum pool from two patients (1:1), Messrs. Perk and O'Rourke. 12-day germfree White Leghorn chicks (Highview stock) inoculated as follows:

#703 inoc. IM @ 0.2 ml ultrafiltrate
710 " " " 0.15 " "
705 " IV " 0.25 " "
708 " " " 0.4 " "

And six contact and diet control birds in same germfree unit, not inoculated.

Results with Exp. No. 61L1-2 to date: Unit is still germfree, 30 days after inoculation. All of the birds are normal and healthy in appearance, and show no weakness on handling. No palpable tumors, nodules, etc.

Table IX.- Gross effect on the thymus nodules of germfree and conventional chickens* 56 days after inoculation with filtrates of pooled sera from patients** with Hodgkins disease.

Chicken No.	Status	Inoculation data			Gross observations	
		Inoculum	Route	ml	Outward appearance	Thymus nodules
706	GF	Ultrafiltrate	IM	0.3	Normal	Normal
709	GF	"	IM	0.25	Normal	Enlarged, Inflamed
708	GF	"	IP	0.25	Normal	Enlarged, Inflamed
710	GF	"	IV	0.2	Normal	Enlarged, Inflamed
707	GF	Sterile water	IM	0.3	Normal	Normal
781	Conv.	Ultrafiltrate	IV	0.2	Normal	Enlarged, Inflamed
785	Conv.	Coarse Filtrate	IM	0.25	Normal	Enlarged, Inflamed
786	Conv.	Coarse Filtrate	IM	0.25	Normal	Normal (?)
787	Conv.	" "	IM	0.25	Normal	Enlarged, Inflamed
782	Conv.	" "	IP	0.25	Normal	Enlarged, Inflamed
783	Conv.	" "	IP	0.25	Normal	Enlarged, Inflamed
784	Conv.	" "	IP	0.25	Normal	Enlarged, Inflamed
788	Conv.	" "	IV	0.2	Normal	Enlarged, Inflamed
789	Conv.	" "	IV	0.2	Normal	Enlarged, Inflamed
601	Conv.	Contact	--	--	Normal	Normal (?)
602	Conv.	Contact	--	--	Normal	Enlarged, Inflamed
603	Conv.	Contact	--	--	Normal	Enlarged, Inflamed

*N.B.- All of same clutch, White Leghorns, Highview stock, 12 days old at time of inoculation. The germfree unit became contaminated with Penicillium mold at 45 days after inoculation.

**N.B.- Dr. A. Rottino's patients, Messrs. Mobilia and Thompson. Serum ratio 1:1.

E. Lymphomatosis:

Our objective in this study* is twofold: (1) to observe the action of the filtrable agent on germfree chicks, and (2) to observe for egg-transmissibility of the causative agent to (otherwise) germfree chicks. Our endeavors to launch this program have proved notably unsuccessful.

To strengthen the probability of incidence under germfree conditions we felt it wise to resort to embryonated eggs from susceptible stock. Consequently, we have made seven attempts**, thus far, to obtain hatch (under germfree conditions) of eggs from inbred susceptible lines developed by the Regional Poultry Research Laboratory. These are fragile, thin-shelled eggs. The developing embryos are killed by our standard germicidal method of transfer into the germfree system. This has necessitated trial modifications in our transfer technique, with resultant higher contamination rates at hatching.

For purpose of record, we make the following report:

In our Experiment No. 56L1-1, eight of eleven 4-day germfree White Leghorn Chicks of Highview stock were inoculated IM and IP with coarse and medium filtrates of tumor material secured from previously inoculated supposedly otherwise germfree birds. However, a contaminant was introduced with this inoculum. The chicks were carried for 35 days, at no time showing illness.

The next five experiments (No. 56L1-2 through 56L1-2), with Regional Poultry eggs, were lost to us either because of exceptionally poor hatch or because of contamination at hatching. Each of these experiments entailed the preparation and setting of 160 or more fertile eggs.

Experiments No. 56L1-6 and 56L1-7 furnished two and five germfree chicks (Regional Poultry), respectively, which were duly inoculated and were thereafter maintained (otherwise) germfree for periods of 147 and 14-21 days, respectively. The results of these two experiments are presented in ensuing paragraphs.

In a final experiment, No. 56L2-1, a unit of eight germfree chicks of Regional Poultry stock was set aside for observation for possible egg-transmission of the virus from the parent stock, in which lymphomatosis is endemic. However, this unit showed bacterial contamination 2-3 weeks after the chicks were hatched.

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* N.B. - In collaboration with Drs. A. M. Lucas, B. R. Burmester, and G. E. Cottral of the Regional Poultry Research Laboratory, East Lansing, Michigan.

** N.B. - Eight germfree experiments in toto, but the first (No. 56L1-1) was made with Highview Farms stock.

Experiment No. 56L1-6: Two 7-day germfree Regional Poultry chicks were inoculated pectorally with an ultrafiltrate of a lymphomatotic liver. These birds were maintained germfree for 147 days post-inoculation, and were sacrificed and necropsied at 150 days. Nineteen 7-day conventional siblings were also inoculated, two with the ultrafiltrate and the remainder with a coarse filtrate of the same liver suspension. The conventional birds were posted at different times from 2 to 7 months following inoculation. With all these birds (germfree and conventional), hematological patterns, including red and white cell counts, hemaglobin, sedimentation rate, packing, and differential counts, were obtained.

Experiment No. 56L1-7: A total of five 8-day germfree and 28, 8-day conventional chicks (all Regional Poultry White Leghorns) were used, as follows:

2	GF chicks inoc.	IM	&	ultrafiltrate of diseased	liver	
2	" "	IP	"	"	"	"
1	" chick (in same GF unit) not treated					
2	Conv. chicks inoc.	IM	&	ultrafiltrate of diseased	liver	
2	" "	IP	"	"	"	"
8	" "	IM	"	homogenate	"	"
9	" "	IP	"	"	"	"
8	" " (in same pens) not treated.					

The germfree birds became contaminated between 14 and 21 days after inoculation. Thereafter these were maintained together with the conventional group. Members of both groups were posted at periods ranging from 1 to 6 months post-inoculation. In many instances, the blood pattern was obtained.

Summary of results with Exps. No. 56L1-6 and 56L1-7: The two birds maintained germfree for 147 days after inoculation failed to give any evidence of incidence, showing no symptomology, a normal blood pattern, and no gross pathology. We have had the personal guidance and assistance of Dr. Alfred M. Lucas in the overall gross and microscopic examination of these birds, but particularly as regards his methods for lymphoid response. His examination of the pancreas, liver, spleen, and lungs, in this respect, has led him to conclude that "none of the reactions were sufficiently convincing to be classed as positive lymphomatosis."*

With the conventional birds, the most commonly observed response pattern was poor equilibration and stupor; on necropsy, many such birds, though not all, showed liver enlargement with or without fatty infiltration, non-specific mottling, or irregular discoloration. Abnormal conditions were found on occasion (no consistent trend) in the pancreas, spleen, kidney, gizzard, gall bladder, intestine, lungs and mesentery. One bird showed numerous gross whitish foci throughout the liver. Another was found to have a tumor-like mass lodged in the pectoral muscle. No definite sign of neural swelling or neural lesions was observed. No trends correlative with the pathological findings are apparent from our rather extensive blood survey data.

Lameness, usually associated with a chondromatous-like condition of the first distal leg joint, was observed in many of these chickens, including one of the long-term germfree birds. However, in all instances, the condition appeared at a very early period and would seem rather attributable to the chicken stock (inbreeding) than to the inoculation.

*N.B.- Personal communication, 23 July 1953.

Plainly, our inoculated conventional controls became, or were, sick birds. Did we observe manifestations of the lymphomatosis agent? Chickens on other experiments, in adjacent pens, showed no comparable effects. The latter, however, were not of Regional Poultry stock. Thus, the somewhat inconclusive nature of our results with this agent to date becomes evident.

We would temper this conclusion with the following speculation.

Our study of published papers on the disease, or diseases, of fowl covered by the term "lymphomatosis" suggests to us that the actual etiological aspect probably is complex. In our investigation of this disease under germfree conditions, we are necessarily limited to the filtrable agent (or agents) involved. Hence it is not inconceivable that syndromic and pathologic patterns evolved with germfree inoculations may not match in all detail the picture found in natural infections or in conventional birds inoculated with tissue homogenates or coarse (cell-containing) filtrates -- or even with ultrafiltrates, so long as the highly influential epidemiologic features of this disease are allowed to play a role.

Our present data, though meagre in the extreme, might be considered to favor the possibilities inherent in this speculation. On the other hand, the potentialities in this regard of the simplest modifications in filtration technique cannot be ignored. If the disease is due to a single and filtrable agent, then the right filtration method should of course obviate any possible discrepancies between germfree and conventional observations, so far as the physiological nature of the agent is concerned.

F. Summary; Tentative Deductions and Conclusion:

We have tried throughout this "Virus Report" to link our several current studies to a common theme, "the comparative physiologic response of the germfree and the conventional animal". We have presented pertinent aspects of our observations to date on the response of germfree chickens to human infectious hepatitis, Hodgkins disease, lymphomatosis, autocleved methylcholanthrene in benzene, and filtrates of methylcholanthrene-induced tumors.

The following would seem to be the more tenable indications in and deductions from our incomplete data:

(1) Tumors may be induced in germfree chickens by methylcholanthrene inoculation. (As with conventional birds).

(2) To date, tumorigenesis has not been observed in germfree, or conventional, chickens following inoculation with ultrafiltrates of MChA-induced tumor tissue.

(3) There may be evidence (a) that response to acute phase human infectious hepatitis sera may sometimes be elicited in germfree chickens; also, (b) that, because of the unreliability of such response, the germfree chicken will probably turn out not to be a highly suitable laboratory host for this human virus. (Probably not unlike the conventional bird?)

(4) Tentative note may be made of a possible pathologic effect on the thymus chain, in both germfree and conventional chickens, following inoculation with ultrafiltrates of sera from patients with Hodgkins disease (Very few observations so far).

(5) Our results thus far with the filtrable agent of lymphomatosis in germfree chickens are inconclusive.

We offer the tentative conclusion that Time will find the germfree and the conventional chicken, in their response to exogeneous agents, to be more remarkably similar than dissimilar.

VII. COLLABORATIVE PROJECTS

Project No. 1 - Dental Caries (with Dr. J. Blayney and Dr. F. Orland of Zoller Memorial Clinic, University of Chicago). (See Section IV - Bacteriology for Report)

Project No. 2 - Studies on the Comparative Effects of Total Body X-Radiation (with the LOBUND-AEC-CWR Advisory Committee). (See Section V - Physiology and Pathology for Report).

Project No. 3.- Investigations on the Influence of the Intestinal Flora on the Infectivity and Pathogenicity of E. histolytica. (With Dr. W. Wright, Dr. C. Rees and B. Phillips of the Microbiological Institute at NIH). (See Section V - Physiology and Pathology for Report).

Project No. 4 - The Study of Hemorrhagic Liver Necrosis Using Germfree Animals (with Dr. Paul Gyorgy and Dr. Martin Forbes of the University of Pennsylvania). (See Section III - Biochemistry and Nutrition for Report).

Project No. 5 - The Role of Ascorbic Acid (or Antibiotics) Replacing the Rat's Requirement for Pantothenic Acid (with Dr. F. Daft and Dr. E. Hawk of the Institute for Arthritic and Metabolic Diseases at NIH). (See Section III - Biochemistry and Nutrition for Report).

VIII. SUMMARY

I. Administrative Section

A review of the changes in staff personnel is given. Alterations in the physical plant are listed. The present status of proposals and contracts is discussed and Capital items received as GFE are listed.

II. Germfree Production, Apparatus and Technique Section

This section is divided into three subsections:

A. Germfree Operations lists the equipment used during the period reported and describes any changes in technique or apparatus which have been made.

B. Germfree Mammal Production gives a resume of the germfree mammal situation during the past six months. It also discusses plans for reorganization in this section.

C. Plastic Garment Sterilization reports on the problem of garment sterilization and advances which have been made in this technique during the past six months.

III. Biochemistry and Nutrition Section

In spite of a decrease in personnel and a deficiency in available germfree cages during the past 6 months, the work of the biochemistry laboratory shows much progress. The relationship of certain B-vitamins has been determined to show that the B-vitamins of the diet become concentrated in the cecum, muscle, brain and especially in the liver while the blood levels are comparatively low. The initial biochemical survey of the germfree rat indicate no major differences between the germfree rat and the conventional rat. A comparison of our data on the composition of rat milk with that of other investigators is presented. The overall picture gives a rather complete characterization for the first time. The services of the diet laboratory with a time analysis of the work is presented. A nutrition study with germfree rats is completed and the data are being processed. The rearing of germfree turkeys presented no difficult technical problems, so a new species of germfree animals has been reared successfully. Collaborative problems underway indicate that an experiment is set up to determine the role of ascorbic acid in riboflavin deficiency and that germfree rats contract liver necrosis when fed the necrogenic diet.

Thus, bacteria are not directly involved in the production of dietary necrosis of the liver.

IV. Bacteriology and Serology

A series of Pseudomonas contaminations was reported in attempted germfree chicken experiments during the 1 January - 30 June 1953 period. A change from 2% mercuric chloride to a quaternary ammonium-type compounded product, "Detergent Sanitizer No. 115"(Rohm and Haas) for use in egg shell disinfection has eliminated Pseudomonas contamination in most recent hatches.

Attempts have been made to sterilize dry whole diets, dietary constituents and antibiotics by methods other than conventional autoclaving or dry heat treatment of the four methods tried: a) ethylene oxide gas; b) alcohol-heat; c) soft x-ray irradiation; d) cathode electron radiation; only the latter has shown successful sterilization in our experience.

Rats maintained in presence of a single type organism, Lactobacillus #465 did not show typical carious lesions observed in conventional controls. One of 4 rats in the lactobacillus group showed enamel (but no dentine) involvement on microscopic examination of the molars.

A similar experiment involving Streptococcus liquefaciens showed peculiar lesions by gross examination in the central sulci of the molars as if the process had been arrested. More detailed microscopic examination is underway. Control rats showed the usual type carious lesions on gross examination.

Currently a similar experiment is underway involving rats harboring only 2 bacterial forms: 1) an acidogenic streptococcus; 2) a proteolytic rod.

Experiments with conventional rats are now being run to establish:

1) Cariogenesis in rats maintained in Quonset colony on a cariogenic diet.

2) Cariogenesis in rats fed 1% Abietic Acid in a cariogenic basal diet.

Bacterial counts were run on swabbings from the oral cavity of rats maintained with a monoflora of Streptococcus liquefaciens as well as in conventional control groups. Special mention is made of the "monoflora" group of rats where oral cultures showed bacterial variation in S. liquefaciens through loss of proteolytic activity in some of the isolates. However, the proteolytic strains predominated.

Rats harboring monofloras of Lactobacillus #465 and Streptococcus liquefaciens #539 produced agglutinins against the homologous organisms (no injection procedures used). Agglutinin titers were stronger in the latter group. The lactobacillus control groups failed to show agglutinins for Lactobacillus #465 but agglutinins were produced against S. liquefaciens in its respective control groups.

Germfree and conventional laboratory reared chickens responded similarly in terms of antibody production to parenterally injected Salmonella pullorum bacterin or bovine serum.

V. Physiology and Pathology Section

1. Quantitative anatomical data originating from germfree and conventional chickens have been statistically analyzed by two methods of grouping. Both methods produced identical characteristics for the investigated animal categories.

2. Variability analysis has shown that the relative weight of "external milieu" organs of germfree chickens is by and large more uniform than in the comparable conventional controls. No such difference was found in the "internal milieu" organs of the two opposing animal categories.

3. Leukocyte balance studies performed in recently contaminated germfree animals show some details of reticuloendothelial response.

4. The amebiasis project, carried on in collaboration with NIH, shows prospects of successful completion in the future, especially since many technical obstacles have been obviated. Details of this project will be summarized later.

5. The study of the effects of x-irradiation (in collaboration with AEC) will be summarized in a special report.

6. Observations were made in autopsied germfree animals lost from the LOBUND colony.

VI. Virology

Description is made of our current studies on the response of germfree chickens to human infectious hepatitis, Hodgkins disease, lymphomatosis, autoclaved methylcholanthrene in benzene, and filtrates of methylcholanthrene-induced tumors. The more tenable implications of our incomplete results are indicated. No unusual differences in response between germfree and conventional chickens may be noted.

VII. Collaborative Projects Section

The five major collaborative projects now being investigated under this Task Order are listed and progress reports are referred to in other sections.